Sleep Disorders in Fragile X Syndrome

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ABSTRACT

Background: Fragile X syndrome (FXS) is the most common genetic disorder associated with mental retardation. Prevalence of FXS is about 0.9 per 1000 among Egyptian school age males. Objectives: To evaluate neurological, behavioral, and neurophysiological dysfunction of children with FXS to improve the quality of life. Methods: Sixteen male patients with FXS (Group 1) selected on basis of their DNA analysis and 16 age and sex matched normal controls (Group 2) were subjected to DNA testing using polymerase chain reaction (PCR) for the diagnosis of FXS, Fragile X check-list, clinical assessment, WISC-III, Childhood Autism Rating Scale (CARS), Electroencephalography, Pediatric Daytime Sleepiness Scale (PDSS), Sleep Questionnaire, and Polysomnographic recording. Results: All FXS patients had delayed language development. 43.75% (n=7) of patients in group 1, scored above 30 in CARS. 50% had epilepsy and EEG abnormalities. The commonest seizure type was partial seizures. Polysomnographic analysis of sleep revealed decreased total sleep time in FXS patients. Analysis of sleep stages showed prolonged stage 2, decreased stage 4 and reduced percentage and number of REM stage in FXS patients. FXS group had poorer sleep efficiency, more awakenings and increased total number of sleep spindles in stage 2 and was associated with higher incidence of epilepsy. Arousals were more in FXS (Number and index of respiratory arousals). Conclusion: Autism and FXS is a common comorbidity. Seizures are common in FXS patients. The NREM sleep alteration found in FXS patients associated with reduction in REM sleep percentage seems to be a distinguished feature that leads to intellectual disability and epilepsy. (Egypt J. Neurol. Psychiat. Neurosurg. 2009, 46(2): 445-454)

Key words: Sleep, Fragile X syndrome, Polysomnography, cognition.

INTRODUCTION

Fragile X syndrome, an X-linked dominant disorder with reduced penetrance. It is associated with intellectual and emotional disabilities ranging from learning problems to mental retardation and from mood instability to autism. Patients suffer from mild to severe cognitive impairment and are characterized by various physical abnormalities.¹

Fragile X syndrome is caused by mutations in a single gene on the long arm of the X chromosome (Xq27.3). It is most often caused by the transcriptional silencing of the Fragile X Mental Retardation 1 gene, (FMR1 gene), due to an expansion of a CGG repeat found in the 5'-untranslated region. The FMR1 gene product, Fragile X mental retardation protein, FMRP, is a selective RNA-binding protein that negatively regulates local protein synthesis in neuronal dendrites. In its absence, the transcripts normally regulated by Fragile X mental retardation protein, are over translated. The resulting over abundance of certain proteins results in reduced synaptic strength due to AMPA receptor trafficking abnormalities that lead, at least in part, to the fragile X phenotype.¹

The prevalence figures for the fragile X full mutation, range from 1 in 4000 to 1 in 6000.²,³ The prevalence of affected males in cohorts of children with special needs ranges from 0.02% to 3%.⁴,⁵ The range may be explained by the populations studied and the diagnostic selection criteria used (e.g., special education classroom, autism, nonsyndromic mental retardation). Whereas the prevalence of FXS mutation among School-Age Egyptian males is 0.9 per 1000. Moreover, the prevalence of FXS is 6.4% among mentally subnormal males.⁶

The most common consistent phenotypic picture of FXS includes mental retardation, which varies from mild to severe, associated with hyperactivity and poor eye contact, subtle dysmorphic features including long face, prominent
mandible, large ears, and macroorchidism in post-pubertal males, hyper-extensibility of metacarpophyaryngeal joints, pectus excavatum, mitral valve prolapse and strabismus.\textsuperscript{7}

Epilepsy is reported in 10–20\% of cases.\textsuperscript{8} Sleep EEG showed no characteristic features. Sleep disturbances are common in FXS.\textsuperscript{10} MRI changes showing decreased grey matter, increased white matter and increased ventricular volume.\textsuperscript{11}

The aim of this work is to evaluate the neurological, behavioral, and sleep dysfunction of children with fragile X syndrome in order to reach easier prognostic scales to help to improve the quality of life.

**SUBJECTS AND METHODS**

**Subjects:**
This case controlled study was carried out on sixteen male patients with fragile X syndrome (Group 1) from 15 different families selected on basis of their DNA analysis among the children attending the outpatient clinic of children with special needs in the National Research Centre (NRC). Their ages ranged from 6 to 18 years with a mean of 10.81± 3.58. The study also included 16 age and sex matched normal controls (Group 2) selected among the children and relatives of working staff. Their ages ranged from 7 to 18 years with a mean of 11.53± 3.27.

**Methods:**
All cases were subjected to the following:
1. A written consent form.
2. Molecular genetic testing: The diagnosis of fragile X syndrome was based on the detection of an alteration in the FMR1 gene. This was based on DNA testing using polymerase chain reaction (PCR). Molecular genetic testing denoting increased trinucleotide repeats and methylation changes in FMR1 gene.
3. Fragile X checklist: A 13-item checklist that combines physical and behavioral traits typical of fragile X.\textsuperscript{12}
4. Thorough clinical assessment.
5. Neuropsychological assessment I.Q: Intelligence Quotient (I.Q) estimates were obtained using the Wechsler Intelligence Scale for Children—Third Edition Revised WIC III. The IQ testing was performed before the sleep study at the initial evaluation. Moderate mental retardation (corresponding to I.Q. in the range 40–55) and severe mental retardation (I.Q. in the range 25–40).\textsuperscript{13}

6. Childhood Autism Rating Scale (CARS): CARS is one of the most widely used scales to evaluate the degree and profiles of autism in children and to distinguish them from developmentally handicapped children who are not autistic. CARS consists of 15 items, each item scores from 1.0 (normal) to 4.0 (severely abnormal) in units of 0.5. The total CARS score is obtained by summing up each item score and thus ranges from 15.0 to 60.0.\textsuperscript{14}

7. Pediatric Daytime Sleepiness Scale (PDSS): The PDSS is an 8-item questionnaire for evaluating the relationship between daytime sleepiness and school-related outcomes. The PDSS was completed by the parent or legal guardian of the patient; scores were considered in the pathological range if above 16.\textsuperscript{15}

8. Sleep Questionnaire:
A simple questionnaire for sleep complaints was given to the caregiver\textsuperscript{16}, usually the mother.

9. Electroencephalography:
EEG was performed using 10 channels Nihon Koden Neuropack. The 10-20 international system was applied. Different montages were used, for example bipolar and monopolar montages, without provocation.

10. Polysomnographic recording (PSG):
The PSG was carried out for patients and for controls, over night using Schwarzer Epos 32 GmpH, medical diagnostic equipment polysomnogram, Germany at the clinical Neurophysiology Unit, Kasr El-Aini Hospital. Sleep stages were scored according to the standard scoring system for sleep stages done by Rechtschaffen and Kales.\textsuperscript{17} The record is scored manually for the following sleep parameters:
   1) Total sleep time (TST).
   2) Percentage of each stage in total sleep time (S1, S2, SWS & REM).
   3) Sleep efficiency (TST/time in bed x 100).
   4) Sleep period 5) Percentage of sleep period.
   6) Number of awakenings.

Arousal index (Number of arousals per hour): An arousal was scored when abrupt shift to a faster EEG frequency occurred and lasted 3–15 seconds.
The numbers of each arousal per hour of sleep were termed the arousal index (AI).

* **Attacks of apnea (central and obstructive apneas):**
  A sleep apnea event was detected when a 10 seconds interval of the signal dropped below 10% of the reference amplitude.

* **Hypopnea detection:**
  A hypopnea event was detected when a 10 seconds interval of the signal dropped below 70% of the reference amplitude.

* **Apnea/Hypopnea index is measured.**

* **Oxygen desaturation events/hour:** Number of oxygen desaturation events /TST x 100.

* **Snoring detection:** Minimum numbers of snores needed to create a snoring period are 3.

* **Periodic limb movement index:** A PLM index above 5 per hour was considered pathologic.¹⁷

**Statistical Analysis:**
1. The arithmetic Mean and Standard Deviation (SD).
2. Comparison of Means using Students’ “T” test.
3. Pearson's Correlation: was used to detect if change in one variable is accompanied by changes in the other value. Significance of Results: Non Significant if P>0.05, Significant if P<0.05 and Highly Significant if P<0.01.

**RESULTS**

**Clinical Data:**
(1) Gross motor development: In (group1): 25% (n=4) gave history of delayed head support, 12.5% (n=2) gave history of delayed sitting, 6.25% (n=1) gave history of delayed walking till the age of 2. None of the patients in group 2 gave history of any delayed motor milestones. (2) Language: 100% of group (1) (n= 16) had history of delayed first word. 93.75 % (n=15) started to acquire language skills after the age of 3 years while only 6.25% (n=1) failed to acquire language skills till now. (3) Social/personal development: Remarkably 100% of the cases (n=16) had history of poor scholastic achievement.

**Intelligence Quotient (IQ):**
In (group 1), IQ ranged from 29 to 78, with a mean value of 61±12.76 SD. In (group 2) the IQ ranged from 80 to 101, the mean value is 89.6±5.74 SD. There is a statistically significant difference between the two groups regarding IQ (p value <0.001).

**Clinical Features of fragile X group (group 1):**
All patients selected in group 1 scored ≥ 16 in fragile X checklist.

**Dysmorphism:** Subtle dysmorphic features: illustrated in figure (1):
- **Face:** 56.25% (n=9) of group 1 had long face.
- **Ears:** 87.5% (n=14) of group 1 showed large cupped ears.
- **Eyes:** upward slanted palpebral fissures in 12.5% (n=2).
- **Nose:** 6.25% (n=1) had broad nose and 6.25% (n=1) had long philtrum.
- **Hair:** None of the patients showed any abnormal characters in hair.
- **Mouth:** 37.5% (n=6) of patients in group 1 showed prognathism. 37.5% (n=6) of patients in group 1 showed thick lips, and 81.25% (n=13) of patients in group 1 had high arched palate.
- **Joints:** 93.75% (n=15) of patients in group 1 showed hyper extensible joints of both upper limbs and lower limbs.
- **Limbs:** 31.25% (n=5) of patients in group 1 showed broad hand and feet with flat feet.

**Behavioral traits:**
- Autistic features: Using child autism rating scale (CARS), 43.75% (n=7) of patients in group 1, scored above 30 in the scale.

**Neurological Examination:**
Neurological examination of group 1 and 2 showed no abnormalities in the motor, sensory, cranial nerves or coordination assessments.

**Other systems Examination:**
- **Heart, Chest and Abdominal examination:** revealed no abnormality detected.
- **Genitalia:** In group 1, 37.5% (n=6) showed shawl scrotum, 31.25% (n=5) showed macroorchidism, 12.5% (n=2) showed hyperpigmentation.

**Epilepsy:**
In this study, FXS patients (group 1), showed that 43.75% (n=7) had normal EEGs and no seizures; 6.25% (n=1) had only EEG anomalies without seizures, 50% (n=8) had EEG anomalies and seizures.
Epileptic seizures:
In group 1, 50% (n=8) gave history of epileptic seizures. All seizure patterns reported occurred during daytime. 18.75% (n=3), gave history of generalized tonic clonic seizures. 31.25% (n=5) presenting with complex partial seizures. None of the patients reported any types of auras and none gave history of febrile seizures. All of the patients are on medications (either Valproic acid or carbamazepine) and the seizures are controlled. None of the cases reported intractable epilepsy.

EEG findings:
In group (1), our subjects performed more than one EEG. 50% (n=8) of our patients showed evidence of EEG abnormalities in the form of centro-temporal sharp waves in 25% (n=4), generalized spike and slow wave in 18.75% (n=3), diffuse dysrhythmia in 6.25% (n=1) and bilateral focal epileptogenic dysfunction in 6.25% (n=1) (Fig. 2).

Neuroimaging:
Conventional CT and MRI showed no detectable abnormality in 87.5% (n=14) while 6.25% (n=1) showed arachnoid cyst in the left temporal region and 6.25% (n=1) showed hypogenesis of corpus callosum.

Pediatric Daytime Sleepiness Scale (PDSS):
In group 1, 100% (n=16) of patients scored 16 and above.

Polysomnographic findings
Overnight PSG was performed for all subjects. Its duration ranging from 6 to 8 hours. All sleep parameters were scored.

I) Sleep Architecture:
Total sleep time (TST):
In Group 1 sleep period ranged from 175 to 445 minutes with a mean of 309.29± 77.83 while in group 2 ranging from 315 to 514.7 minutes with a mean of 404.17±100.48. A Statistically significant difference was found between group 1 and 2. TST was lower in group 1 (P value < 0.001) Sleep onset: In Group 1 sleep onset ranged from 0 to 45.9 minutes with a mean of 8.78± 13.61 while in group 2 ranging 0 to 21 minutes with a mean of 10.87±4.68No Statistically significant difference was found between group 1 and 2 Sleep period: In Group 1 sleep period ranged from 182.5 to 521.6 minutes with a mean of 361.64±100.48, while in group 2 ranging from 347 to 530.7 minutes with a mean of 439.69±57.06. Sleep period was significantly lower in group 1 than group 2 (P value = 0.01).

II) Sleep Stages
Percentage of Sleep stages: Table (1).
In light sleep, a statistically significant difference was found between group 1 and group 2 in (stage 2) higher values were reported in group 1 (P value= 0.004) but no statistically significant difference was found between groups in (stage 1).

In deep sleep, a statistically significant difference was found between group (1) and group (2). In (Stage 4), lower values were reported in group (1) (P value = 0.02) but no statistically significant difference was found between groups in (Stage 3).

In REM sleep, a highly statistically significant difference (P value< 0.001**) was found between group 1 and 2. REM was significantly lower in group 1.

In REM periods:
A Statistically significant difference was found between group 1 and 2 in the number of REM periods it was higher in (group 2) with a mean of 5.86±1.24 than (group 1) with a mean of 0.69±1.3 (P<0.001) and also in the longest REM period as it was also higher in group 2 with a mean of 32.62±11.5 than group 1 with a mean of 8.84±13.22 (P<0.001).

Arousal:
A Statistically significant difference was found between group 1 and 2 in the number of arousals and respiratory arousals. Both number (mean of 2.63±2.8) and index of respiratory arousals (mean of 0.5±0.54) were higher in group 1 (P value = 0.001) compared to group 2 who reported no respiratory arousal (Table 2).

Spindles:
A statistically significant difference was found between group 1 and 2 in the number of spindles, especially number of spindle in stage 2 of sleep was higher in group 1(1999.56±910.2) than group 2 (349.3±242.63) p value <0.001
Apneas:
A Statistically significant higher values were found in group (1) than group (2) in the number of apneas, number of apneas/ hour, (P value <0.001) and longest apneas in seconds (P value =0.003). There was also a statistically higher significant difference in the apnea/hypopnea index per hour in group (1) as compared to group (2) (P value =0.001).

Obstructive apneas:
A Statistically significant higher values were found in group 1 with a mean of (6.44±5.15) than group 2 with a mean of (1.6±2.1) in the number of obstructive apneas during sleep (P value= 0.002).

Central apneas:
A Statistically significant higher values were found in group 1 with a mean of (3.63±2.47) than group 2 with a mean of (0.44±5.15) in the number of central apneas during sleep (P value=0.02).

Hypopneas:
A Statistically significant difference was found between group 1 with a mean of (47.62±17) and group 2 with a mean of (73±23.43) in the percentage of hypopneas during sleep (P value=0.001).

Oxygen desaturation events:
There were no statistically significant differences between group 1 and 2 regarding Oxygen desaturation events during sleep P value 0.5 or lowest O2 saturation during sleep P value 0.9.

Periodic Limb Movements Statistics:
Limb Movements with Apnea Hypopnea index was significantly lower in group (1) (P value= 0.002) while limb movements with no associations was significantly lower in group 1 than group (2). There was no statistical significance between the groups in Limb movement index or in the Limb movement with arousal index. P value 0.26.

Awakenings
The number of awakenings in group (1), was higher than that of group (2), (P value = 0.01) but the index was not statistically significant. P value 0.01*

Correlative Studies
* Correlation between IQ and polysomnographic parameters:
Obvious inverse relations were shown between the total sleep time & the intelligence quotient, yet they did not reach the level of statistical significance (P value > 0)

* Correlation between CARS and polysomnographic parameters:
No statistical significant difference was shown in our results correlating the CARS and the polysomnographic parameters.

Fig. (1): Distribution of subtle dysmorphism in (group 1).
**DISCUSSION**

Fragile X syndrome (FXS) is the most common form of inherited mental retardation, with prevalence of 1:2000. Meguid et al. reported a prevalence of 0.9 per 1000 among School-Age Egyptian males.

In the present study, 94% of FXS patients gave history of delayed speech while only 6% of the patients failed to acquire language skills till now. This is consistent with the findings of Alanay et al., who reported that FXS mutation produces significant impairments in speech and language.

Tassone et al. pointed out that in males with FXS, language development was intimately related to the cognitive development.

The motor development in FXS patients was normal. This is in agreement with Bailey et al.,

![Fig. (2): EEG findings in group (1).](image-url)
who reported that motor development in FXS is better than communication and cognitive skills.

The present work revealed that I.Q. assessment using WISC-III for patients with FXS ranged from 29 to 78, with a mean value of (61±12.76). These findings were in accordance with those reported by Musumeci et al., where it ranged from 25 to 75 with a mean value of (43.91±11.18). 100% of patients involved in this study had poor scholastic achievement. This finding was in agreement with those reported by Robert et al., who observed that boys with FXS had significant deficits in all academic skill areas with relative strength in general knowledge and relative weakness in visuospatial-processing abilities. It has been speculated that in cases with FXS, the number of CGG triplet repeats was inversely proportional to academic achievement in mathematics and English. Hessl et al. mentioned that prevalence of autism in FXS was the same as its prevalence in other causes of mental retardation. This study disclosed that autistic symptoms were present in most of the cases with FXS and it ranged from mild to severe (43 % scored over 30 in CARS). These results were in harmony with previous studies conducted by Freund and Reiss. The association of FXS with epilepsy was first reported by Lubs and subsequently by Sutherland and Hecht. In this study 50% of patients with FXS had epilepsy and 6.25% of the patients had epileptiform activity without clinical seizures. These results were in line with the findings of Musumeci et al., who noted that in patients with FXS, the prevalence of epilepsy ranged from 10 to 40%. On the other hand, Ramos mentioned that FXS patients had only EEG epileptiform abnormalities unaccompanied by clinical seizures. Interestingly, it was observed that the majority of patients with FXS show resolution with late childhood. This may account for the higher prevalence of epilepsy in the present study as the mean age of the participants was 11 years.

In the current study, partial seizures were the most reported type of seizures 31.25%, 19% of FXS patients had generalized tonic clonic seizures. However, previous reports characterizing the type of clinical seizures associated with FXS were rather argumentative. Whereas some authors reported that seizures associated with FXS were usually generalized, others noted that they were predominantly partial (75% and 50%, respectively).

The EEG findings recorded in this work were Centro-temporal spikes (25%), generalized spike and slow wave complexes (18%), asynchronous slowing (6%) and bilateral focal epileptiform dysfunction (6%).

The neurobiological processes underlying CNS dysfunction in FXS appear to result in increased neuronal excitability which is specifically associated with Centro-temporal spikes. Hence, the Centro-temporal spikes pattern characteristic of BCECTS seems to be the phenotypic marker of FXS. In this study, there is a significant decrease in REM sleep, slow wave sleep (stage 4) together with a significant increased duration of (stage 2) of NREM sleep. Moreover most epileptic phenomena were provoked during S2 stage of NREM sleep.

Daytime epileptic seizures are more common in NREM than in REM sleep, especially in the lighter stages of NREM sleep (Stages 1 and 2). NREM sleep is looked as a state of relative “neuronal synchronization”, with coordinated synaptic activity, accompanied by recruitment of a critical mass of neurons needed to initiate and sustain a seizure. This synchronization is associated with neuronal hyperexcitability with a lower convulsive threshold during NREM sleep than during REM sleep and wakefulness. On the other hand, During REM sleep cortical activation occurs when cholinergic brainstem afferents increase firing rates producing a state of hyperpolarization in the thalamocortical relay neurons and consequently, REM sleep is known to be protective against epilepsy.

The present study revealed that, the total sleep time, the sleep periods and the sleep efficiency were significantly lower and the episodes of arousal were significantly higher in the FXS patients compared to controls. These findings go hand in hand with those reported by Holloway et al. On the contrary, Miano et al. reported no statistical difference between FXS patients and controls.

This study also illustrated that FXS patients with epileptic seizures had a higher incidence of NREM sleep with spindles as compared with FXS patients without epileptic seizures this might draw attention to the relationship between the spindle production during NREM sleep and the presence of epileptic seizures. It is worth noting that there is a similarity of generators for spindles and for spike and wave discharges.

In addition, in the present study, both the number of REM periods and their percentage were
significantly lower in patients with FXS as compared to controls. It has been hypothesized that REM sleep is considered to be an index of the brain “organizational abilities” i.e. the ability to organize information from random pool of elements into long term memory.\(^3\) Besides, there is considerable information suggesting that increased REM is correlated with enhanced learning of certain tasks in healthy normal volunteers, which may be correlated with its function.

Additionally, the present work revealed that the percentage of slow wave sleep was significantly lower in FXS patients. It has been speculated that slow wave activity of NREM sleep may be involved in producing progressive downscaling of synaptic strength which in turn could lead to several benefits in terms of cellular function and network performance.\(^4\)

Moreover, the present work underlined the findings of Tirosh et al.\(^5\), who reported a high incidence of obstructive apneas in patient with FXS. It is interesting that the observed sleep apnea might account, in part, for the associated sleep fragmentation as well as the poor scholastic performance.\(^6\)

Finally, it is worthy to mention that, the NREM sleep alterations found in this study, associated with the reduction of REM sleep percentage, are distinctive features of the intellectual disability and Epilepsy in FXS patients. Consequently, this study might underline the usefulness of polysomnography in the comprehension of the neurophysiological mechanisms underlying FXS.

**REFERENCES**


اضطرابات النوم في مرض متلازمة كروموسوم أكس الهش

تعد متلازمة كروموسوم أكس الهش من أكثر الأسباب شيوعاً للتخلف العقلي، يأتي نتيجة من ظاهرة في أحيان الجينات الوراثية التي يمكن أن تنتقل من جيل إلى جيل.

يتزامن مع متلازمة كروموسوم أكس الهش فرق النشاط الحركي، نقص الانتباه وصعوبة التعلم واضطراب النوم مع وجود نوبات صرعية.

تم إجراء هذا البحث على مجموعتين: المجموعة الأولى تتكون من 16 شخص مصاب بمتلازمة كروموسوم أكس الهش.

والجموعة الثانية هي المجموعة الضابطة.

وقد تم في المجموعتين وإجراء الفحوصات التالية: تقييم سريري كامل للجهاز العصبي؛ اختبار ذكاء مقياس مستويات النوم لدى الأطفال، استبان عن النوم ودراسات النوم.

وقد أظهرت نتائج البحث وجود أصل متزايد تشير أن هناك تزامن واضح بين متلازمة كروموسوم أكس الهش وصعوبات التعلم وتأخر اللغة، ووجود أداة إحصائية تشير إلى وجود مرض المسمى في 44% من الحالات وكان الصرف الجزئي هو أكثر الأنواع شيوعاً.

أما نتائج دراسات النوم أظهرت نقص إجمالي وقت النوم وضعف كفاءة النوم واضطراب في نمط مراحل النوم في صورة زيادة في نوم غير الحركة العينية وقصور في مدة نوم حركة العين السريعة، وأظهرت أيضا أن مرض كروموسوم أكس الهش يعانون من زيادة توقف التنفس الادسادي أثناء النوم.

ومما سبق يتبين أهمية تحليل نمط النوم لدى مرضى كروموسوم أكس الهش لما لها من دور في التطور العقلي النفسي.