Azhar Int J Pharm Med Sci 2022; Vol 2 (2): 43-51
Research Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Assessment of antioxidants biomarkers in plasma of fragile X syndrome patients

Rasha S. El-Mahdy[1*], Noha N. Amer[2], Nagwa A. Meguid [1], Adel Hashish [1], Sohair M. Salem [3], Ola S. Ali [2]

[1] Department of Research on Children with Special Needs, Medical Research Division, National Research Centre, Giza, Egypt.
[2] Department of Biochemistry, Faculty of pharmacy (Girls), Al-azhar University, Cairo, Egypt.
[3] Department of Molecular Genetics and Enzymology, National Research Centre, Giza, Egypt.
[*] Correspondence: e-mail: mesque_2004@yahoo.com

Article history: Received: 6-9-2021 **Revised:**25-9-2021 **Accepted:** 24-10-2021

Abstract: Numerous neurological diseases are exacerbated by oxidative stress, including fragile x syndrome (FXS), which occurred as a result of a mutation in the fragile x mental retardation 1 gene (FMR 1-gene). Several studies have found that redox equilibrium is disrupted in the brains of knockout mice with fragile x syndrome, suggesting that antioxidant levels in plasma may reflect the oxidative stress profile in FXS. The goal of this study aimed to evaluate antioxidants biomarker levels in FXS patients indicating that antioxidants can be used as indicators in FXS patients. This study included a total of 60 subjects (30 suspected FXS patients: 15 males and 15 females, while the remaining 30 were healthy subjects). Full blood genomic DNA was used, followed by gel electrophoresis and CGG repeat calculation. Antioxidant biomarkers (total glutathione, glutathione peroxidase, and superoxide dismutase) were measured in the plasma of patients and controls groups. The study revealed that the average levels of antioxidant biomarkers in FXS patients were significantly lower than in controls. FXS and oxidative stress are linked, implying that oxidative stress plays a role in the pathogenic process. Antioxidants may be helpful in the therapy of FXS.

Keywords: Fragile X syndrome; oxidative stress; antioxidants; total glutathione; glutathione peroxidase; superoxide dismutase.

1. INTRODUCTION

One of the most typical reasons of neuroimpairment diseases is Fragile x Syndrome (FXS, OMIM 300624). It is an X-linked disease that affects one in every 4000 males and one in every 8000 females [1]. Patients with Fragile x suffer a variety of hazards, ranging from intellectual disability to the development of behavioral issues such as autism, anxiety, and hyperactivity [2]. The increase of polymorphic CGG repeats element in the 5′ untranslated regions of the Fragile x Mental Retardation-1 gene (FMR 1 gene) is the principal common reason of fragile x syndrome. Over 98 percent of the reported instances are included in the expansion [3]. The protein synthesis of the FMR1 gene, also known as fragile x mental retardation protein (FMRP), is shut off as a result of this abnormal expansion, which causes incorrect methylation of the surrounding 5' upstream CPG island [4].

Because of the effect on FMR1 gene translation, CGG-repeat expansion causes a group of diseases. The non-coding CGG-repeat element has been enlarged to a number of different genetic allelic forms. The permuted allele varied from (55 to 200 CGG repeats), the full mutated allele is over than 200 CGG repeats, and the normal allele contains fewer than 45 CGG repeats. Gray zone alleles have CGG repetitions of 45–54, are considered unstable, and can be passed down to the following generation[5-7] Patients with full mutation have been linked to mild to severe mental deficiency with IQs ranging from 20 to 70 [8].

The main pathologic consequence of this condition is the loss of protein expression or translated product, as the brain is a high-energy-consuming organ compared to other organs [9, 10]. Imbalances in redox homeostasis have been seen in the brains of knockout mice with fragile x syndrome; this imbalance causes common symptoms in this disorder, impacting their progression and severity. This emphasizes the need of measuring antioxidant biomarker activity or concentrations [11].

Cite this article: El-Mahdy R., Amer N., Meguid N., Hashish A., Salem S., Ali O. Assessment of Antioxidants Biomarkers in Plasma of Fragile X Syndrome Patients. Azhar International Journal of Pharmaceutical and Medical Sciences, 2022; 2(2):43-51. doi: 10.21608/AIJPMS.2022.93451.1088

DOI : 10.21608/AIJPMS.2022.93451.1088 https://aijpms.journals.ekb.eg/

When the balance between the creation of reactive oxygen species (ROS), also known as oxidants, and their eradication by antioxidants in biological cells is disrupted, lipids, proteins, and nucleic acids are damaged, finally leading to cell death. ROS is produced by cells of organisms that are alive as a result of regular cellular metabolisms and external variables in low to adequate quantities; but, at high doses of oxidants, ROS causes significant changes to cell components [12, 13].

The ability of cells to proliferate and carry out their fundamental biofunctions is dependent on equilibrium in the oxidative stress state. Endogenous variables (i.e., NADH oxidase activities, and mitochondrial metabolisms), and Exogenous influences (i.e., chemicals or UV radiations) [14]. The mitochondrial metabolism and NADH oxidase activity can be used to determine ROS, as well [15]. In aerobic cells, the major endogenous sources of ROS generation and elimination are tightly regulated[16]. The antioxidant removal systems consist of enzymatic and non-enzymatic antioxidants. They are usually successful in preventing ROS's harmful effects in living cells [17]. This is because aerobic organisms utilize byproducts of oxygen molecules like (superoxide anions, hydroxyl radicals, hydrogen peroxides) for transduction, and O_2 molecules in redox processes required for energy demands. The antioxidant mechanisms, on the other hand, can be overloaded under pathological conditions [12, 14, 18].

Almost every aerobic cell and extracellular fluid contains enzymes; which serves as a guard against the cell damage like superoxide dismutase (SOD) [19], catalase [20], glutathione peroxidases (GPXs) [21], as well as, endogenous defenses include antioxidant systems compounds that are active as scavenge molecules like as glutathione (GSH), vitamin E, vitamin C, uric acid[17] . The activity and expression changes in the antioxidant enzymes in the brain tissues and in Alzheimer's disease, Down syndrome, neurology problems, autism, and repeated expansion of DNA have been documented in numerous studies, notably in the case of superoxide dismutase (SOD). As a result, oxidative stress could be a key pathogenic characteristic in FXS. So enzymatic biomarker of oxidative stress in FXS patients are being assessed in this study [23].

Glutathione is the tri-peptide consisting of (γ-L-Glutamyl-L Cysteinyl Glycine) that is located in all living cells of mammalian tissues and is predominantly abundant in hepatic tissues [21]. The commonest prevalent nonprotein thiol that protects verses oxidative stress is GSH [16]. Glutathione serves as a cellular protector in the brain tissues by trapping highly reactive oxygen species radicals (ROS) and is thus implicated in a variety of cellular survival mechanisms in responses to oxidative stress [21].

Glutathione peroxidase is a group of isoenzymes that use GSH as an electron donor to catalyze the reduction of H_2O_2 and lipid peroxides [24, 25]. Both the cytosol and the mitochondria of cells contain GPX. There are five distinct selenium-dependent isoforms in mammals (GPXs 1 to 4 and 6), and three selenium-independent glutathione peroxidase isoforms (GPXs 5, 7, and 8) [26]. Each isoform and its cell location affect the potential of GPXs to serve as antioxidants. It's worth noting that GPX1 is one of the most essential antioxidants enzymes in the brain cells, and that it's predominantly expressed in microglia rather than neurons[27, 28].

Superoxide dismutase is an enzyme that supports conversion of highly reactive O_2 species to less reactive H_2O_2 and oxygen molecules. SOD exists in three different isoforms: SOD1 and SOD2 are engaged in the cytosol and mitochondrial elimination of O2, respectively, and extracellular SOD is involved in the elimination of O2 (SOD3) [19, 24].

The goal of this study was to assess the activity of (total glutathione, glutathione peroxidase, and superoxide dismutase) as an oxidative stress biomarker in FXS, and to underscore the importance of tracking permuted females' oxidative stress levels in order to forecast the oxidative stress profile that will help the following generation develop.

2. SUBJETS AND METHODS

2.1. Subjects

2.1.1. Subjects group analysis

An average of 300 patients attends Research on children with special needs clinic at National Research Centre annually suffering from learning disability to mental subnormality. From 2015 to 2021 approximately we received 1000 male children. Hagerman checklist was run to all attending patients. Those scoring IQ (20-70) and suffering developmental disability with physical features or behavioral problems suggestive of FXS were selected for FXS molecular testing. Intelligent quotation (IQ) was tested using Illinois test, and Stanford Binnet test; were done for all suspected males, PCR was performed depending on Hagerman's checklist. All 15 male patients that revealed a full mutation FMR1 gene were enrolled in this study. Their ages ranged from 7 to 20 years old. In addition, the fifteen mothers of the enrolled male subjects agreed to participate in this study. Their ages ranged from 27 to 43 years old. Control groups included 30 healthy normal subjects not suffering from any related mental diseases or

subnormality (11 males and 19 females) with matched ages to the suspected males and their mothers. Written consents were obtained from all participants. The present study was agreed by ethics committees of both National Research centre and Faculty of pharmacy (Girls) - Al-Azhar University Egypt (Approval number 93/2016).

2.2. Methods

2.2.1. Collection of samples

A blood sample of 3 ml was obtained from each subject in participant groups, collected on EDTA tubes. After that, each sample was divided into 500ul for genomic DNA extraction, the rest was centrifuged, plasma was separated, and stored at -20 C^o for antioxidant biomarker analysis.

2.2.2 Genomic DNA extraction &CGG repeats calculation

Axygen®DNA extraction kit, manufactured by (Axygen Bioscience cat No: 33210 USA), has been used to extract genomic DNA from peripheral blood samples as per the manufacturer's protocol. CGG reproduces regional elements of the FMR1 gene using special primers PCR (Saluto et al), utilizing primers (forward 5′-agccccccacttccaccaccaccaccaccaccaccacctcca-3″) and (reverse 5′-gctcagctcgctcgettcggtccggt-3″) with minor modifications [29]. To calculate the CGG repeats, 5 µl from each PCR product were electrophoresed at 6 V/cm for 45 minutes on a 2.0% agarose gel in 1X TBE containing ethidium bromide using documentation system (Syngene GeneGenius Bio Imaging System, Cambridge, UK). Unknown PCR bands have been estimated based on software from Gene Tool (Syngene, Cyprus).

2.3. Oxidative stress assessment

2.3.1. Glutathione peroxidase assay

The activity of glutathione peroxidase in the plasma of participants with (full and Pre mutations) as determined by the FXS molecular assay, as well as control subjects, was assessed. Glutathione peroxidase activity was evaluated using a glutathione peroxidase Assay Kit (No. 703102 MI • USA) according to the manufacturer's procedure.

2.3.2. Total glutathione assay

Total levels of glutathione were measured by the use of the manufacturer's procedure for the OxiSelectTM total glutathione (GSSG) test kit Caiman Item (No. STA-312 • USA) in plasma in the individual patients (with full mutation and premutation).

2.3.3. Superoxide dismutase assay

Superoxide Dismutase activity was measured in the plasma of subjects with full and Pre mutations as indicated by the FXS molecular assay. The activity of superoxide dismutase was determined using the Superoxide Dismutase Activity Abnova test kit (No. KA0783, ab 65354).

2.4. Statistical analysis

Statistical analysis was conducted using social science statistical tools, version 20.0. (SPSS Inc., Chicago, Illinois, USA). The contributed quantitative data have been represented as a mean ± standard error. An independent T test was utilized in comparing control and patient group for the normally distributed data. The non-parametric Mann Whitney test has been used to compare non-distributed data. In order to convey Qualitative data frequency and percentage were utilized. Statistically significant was the $P< 0.05$, and considered highly significant at $P<0.01$. Using the Spearman correlation, the correlation between different biochemical markers was evaluated.

3. RESULTS

3.1. Subject Group Analysis

There were 60 individuals included in this study. They were separated into two sets, FXS and controls, each of which was composed of 30 participants. The Hagerman checklist, Stanford Binnet Test, and the Illinois Test clinically assessed the FXS patients. Then a molecular analysis was carried out. Fifteen male patients who did not amplify their samples from 1000 suspicious samples; while other samples were in normal range. The ages were from 7-20 years old for probable male cases. Fifteen additional samples from Mothers of probable FXS males requested to participate in this study, their ages ranged from 27 to 43 years old. A sample of the PCR normal, premutation and full mutation gel electrophoresis was displayed in the figure (1).

3.2. Assay of antioxidant markers

Demographic and biochemical statistical analytical data of the studied groups were illustrated in table (1), with values expressed as number (%) or mean ± SE. Total glutathione, glutathione peroxidase, and SOD in the FXS patient group were significantly lower than in the control group at $p<0,001$ as shown in table (1) and figure (2).

3.3. Gender comparison of antioxidants biomarkers

All male patients were molecularly diagnosed as full mutation, while all mother females were molecularly diagnosed as premutation. The mean±SE plasma levels of total glutathione, glutathione peroxidase and superoxide dismutase were significantly lower in male and female patients than in their comparative control at $P< 0.00$ for males and females. However we noticed that there was a significant difference in the mean±SE plasma levels of total glutathione, glutathione peroxidase in the female patients group compared with the male in the same patients groups, while there was no any

significant difference in the mean plasma levels of the (SOD) between the two different groups. There was no significant difference between female to the male in control group for the three antioxidant parameters, as shown in table (2).

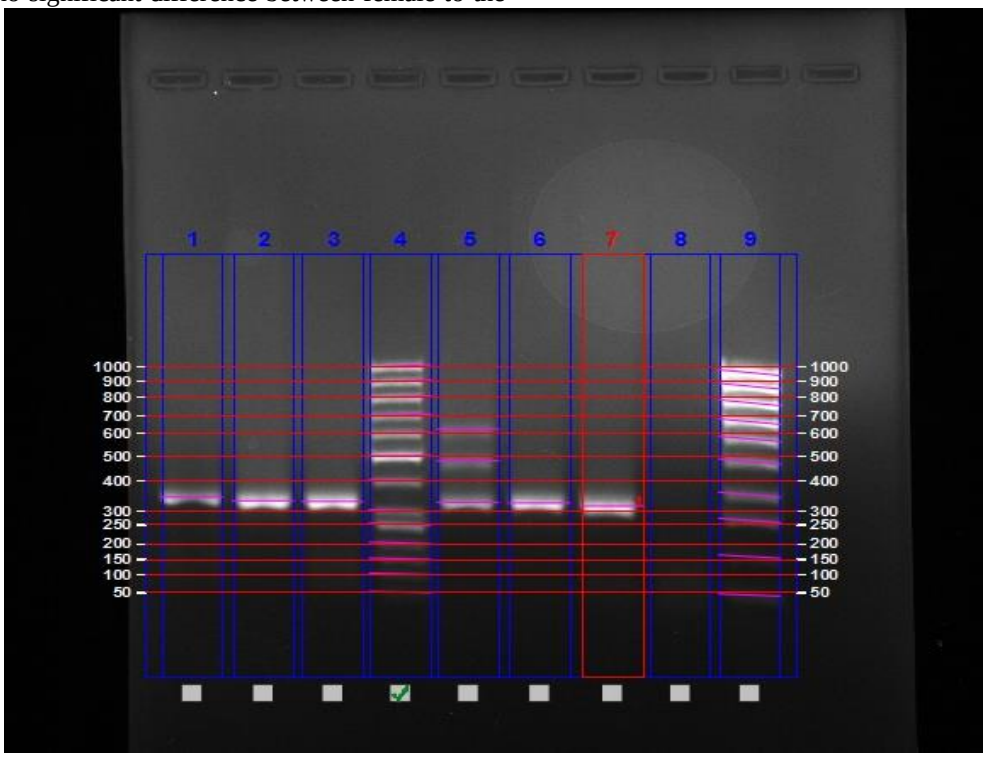

Figure 1. Agarose gel electrophoresis of amplifies DNA samples from different patients.
Lanes 4 & 9 represent DNA molecular weight markers (50-1000 bp). Lanes 1,2,3,6 & 7 represent PCR products of normal unaffected males of (37,34,33,30,31) CGG repeats respectively. Lane 5 represents PCR products of carrier heterozygote pre-mutated female of (93, 125) CGG repeats. Lane 8 shows no amplification of PCR of male patient suspected with fragile X syndrome.

Table1. Demographic and biochemical data of patients and controls.

Characteristic	**control n=30**	**patients n=30**
Demographics		
Sex, n (male %)	11 (36%)	15 (50%)
Age(Y)), mean±SE	25.83±2.091	23.26±2.62
Biochemical parameters		
Total glutathione (µmol/g Hb), mean±SE	6.787±0.4134	3.296±0.303**
Glutathione peroxidase (U/g Hb), mean±SE	41.623±2.388	23.743±1.311**
Superoxide dismutase (Pg/ml), mean±SE	2355.467±176.884	1488.533±142.553**

SOD: Superoxide dismutase.
**Statistically significant difference from control group at $P \leq 0.05$.
** Statistically highly significant differnce from control group at $P \leq 0.01$.

3.4. Correlation between antioxidant biomarkers

In patients, plasma level of total glutathione showed a significant positive correlation with glutathione peroxidase (r=0.614, p<0.000), while it showed no significant correlation with SOD (r= 0.259, p=0.05). However plasma glutathione peroxidase level revealed a significant positive correlation with SOD (r=0.311, $p \leq 0.05$) table (3).

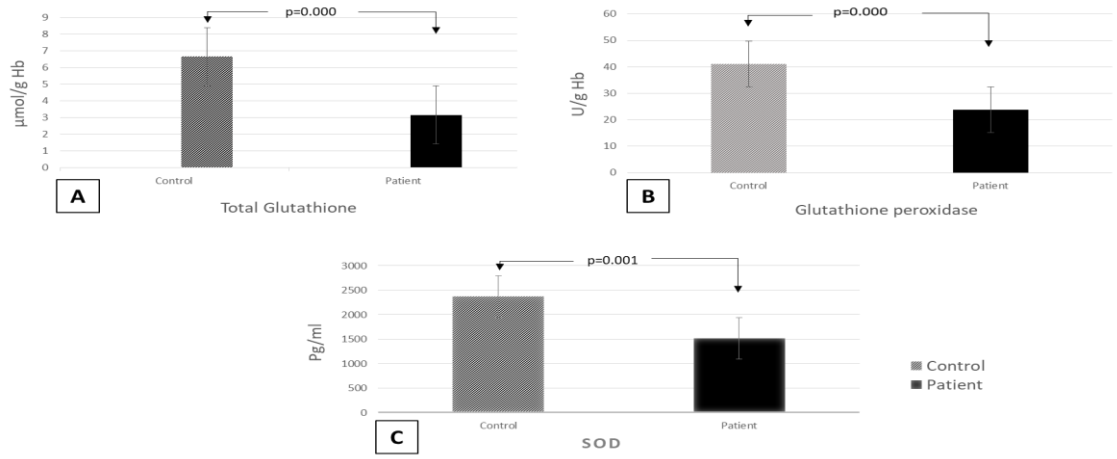

Figure 2. Distribution of relative total glutathione (tGSH), glutathione peroxidase, and SOD in patient and control groups: Mean ± SE of total glutathione (A), glutathione peroxidase (B) and superoxide dismutase (C) in the controls and patients' groups.

Table 2. Comparison between males and females in controls and patients' groups with antioxidant biomarkers.

Biochemical Parameters	***Control***		***Patient***		***P Value***
	Mean ± SE		***Mean ± SE***		
	Males (n=11)	***Females (n=19)***	***Males (n=15)***	***Females (n=15)***	
Total Glutathione (µmol/g Hb)	7.285 ±0.835	6.499 ±0.445	2.879 ±0.465**	3.7126 ±0.373**	Male: 0.000 Female: 0.000
Glutathione peroxidase (U/g Hb)	44.509 ±3.051	39.953 ±3.331	20.54 ±1.198**	26.946 ±2.053**	Male: 0.000 Female: 0.004
SOD(Pg/ml)	2232.909 ±272.732	2426.421 ±234.317	1567.2 ±255.191**	1409.867 ±134.838**	Male: 0.092 Female: 0.001

** Statistically highly significant difference from control male or control females at P < 0.01.

Table 3. Correlation between antioxidant biomarkers in patients' groups

		Total Glutathione	**Glutathione Peroxidase**	**SOD**
Total Glutathione	Correlation Coefficient	1.000	0.614**	0.259
	Sig. (2-tailed)		0.000	0.050
Glutathione Peroxidase	Correlation Coefficient	0.614**	1.000	0.311*
	Sig. (2-tailed)	0.000		0.018
SOD	Correlation Coefficient	0.259*	0.311*	1.000
	Sig. (2-tailed)	0.050	0.018	

* Correlation is significant at p≤0.05, ** Correlation is significant at p≤0.01.

4. DISCUSSION

Fragile X syndrome is a neurodegenerative X-linked disorder characterized by a wide range of cognitive, behavioral, and physical issues[30]. Because the increase of CGG repeat elements in the FMR1 gene locus Xq27.3, hyper methylated area in the gene promoter originated, silencing the FMR1 gene reduces FMRP expression. FMRP is a protein that has a role in synaptic development and plasticity[31].

Increased stress in brain tissues is thought to play a role in cognitive impairment. Alzheimer's disease, Parkinson's disease, Friedreich ataxia, and other mental disorders such as autism, schizophrenia, Down's syndrome, and Fragile X syndrome have all been linked to this impairment [9, 11,23,33,34]. Several investigations have shown that the brain oxidative

stress plays a role in the state of fragile X. El Bekay et al.(2007)identified the oxidative stress pathways in the Fmr1-KO Mice brains, including free-radical abnormalities, increased ROS levels and reduced antioxidant indexes including GSH, GSH peroxidase and GSH transmission. This disequilibrium between the ROS and antioxidants increases oxidative stress in young Fmr1-KO mice's brains which contribute to their phenotype [11]. However, this technique is invasive, and difficult to apply to FXS prognosis.

High levels of oxidative stress biomarkers in the blood indicate similar stress status in brain, according to several studies [9, 23]. The capacity to measure antioxidant levels in systemic circulation has sparked focus in using them as biomarkers for risk assessments and disease severity progressions, which is an important step in evaluating the response to preventative interventions.

The present study revealed that total glutathione, glutathione peroxidase, and SOD were significantly lower than controls group for the patients' group, which is supported by prior studies. Lower SOD levels and glutathione peroxidase had been seen in patients with Alzheimer's disease (AD) [9, 23]. GSH blood levels were identified as a major predictor of cognitive performance in individuals suffering from Alzheimer's disease, with a connection between decreased blood GSH and severe cognitive impairment [33]. In addition, patients with Parkinson's disease showed decreased GSH, a major antioxidant molecule [34].

Our study showed a significant difference in the mean ±SE plasma levels of total glutathione; glutathione peroxidase in the female patients' group compared with the male in the same patients' group, while there was no any significant differences in the mean ± SE plasma levels of SOD between the two groups. There was no significant difference between female to the male in control group for the three antioxidant parameters, these findings indicate a favorable relationship between mothers and their sons. Another study discovered a close correlation between mothers' oxidative stress and those of their neonates, with a high mother's oxidative stress leading to even higher oxidative stress in their children[35] These findings underscore the importance of tracking permuted females's oxidative stress levels in order to forecast the oxidative stress profile that will help the following generation develop.

The present study also demonstrated, that the plasma level of total glutathione was shown to have a significant positive association with glutathione peroxidase in patients' group at $p<0, 00$ (table 3), but not with SOD. The plasma levels of glutathione peroxidase, on the other hand, were shown to have significant positive association with SOD at $p\leq 0.05$ (table 3). The positive association between glutathione with glutathione peroxidase and SOD may be due to the complimentary action between them, since the GSH—is a substrate of glutathione peroxidase. Glutathione peroxidase and SOD contribute to the cascade pathway of action. These findings were in agreement with other studies, which showed significant decrease in the plasma level of total glutathione in concordant with glutathione peroxidase level in several diseases such as pulmonary tuberculosis [36], and anemia [37]. In addition, Lovell et al. (1995) demonstrated significant decrease in glutathione peroxidase in parallel to SOD level in the brain in patients with Alzheimer's disease[38].

5. CONCLUSIONS

The present study reported significant decrease in plasma levels of antioxidant biomarkers in FXS patients compared with control subjects. Antioxidant biomarkers may represent as a corner stone of understanding pathophysiology of FXS. An ideal biomarker would be a step in the right direction that connects the underlying pathology to clinical manifestations of illness. Further studies are required to clarify the role of antioxidant biomarkers in FXS prognosis and progression.

Funding: This research was funded by National Research Centre.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethical Statement: The protocol followed in this study was approved by Al-Azhar Faculty of Pharmacy (Girls) scientific research ethical committee and from National Research Centre ethical commitee.

Author Contribution: This work was carried out in collaboration between all authors. Nagwa Abdel Meguid, Adel Hashish and Sohair Salem designed the study. Rasha Samir El-Mahdy performed the experiments and wrote the manuscript. Noha Nagah Amer, Ola Sayed Ali and Rasha Samir El-Mahdy analyzed the data and revised the manuscript.

List of Abbreviations: FXS: Fragile x syndrome; AD: Alzheimer's disease; SOD: Superoxide dismutase; PD: Parkinson's disease; Fmr1gene: Fragile x menta lretardation 1 gene; KO mice: knockout mice; ROS: Reactive oxygen species; GSSG: oxidised glutathione; GSH: Reduced glutathione; tGSH: Total glutathione.

REFERENCES

1. Lozano R, Azarang A, Wilaisakditipakorn T, Hagerman RJ. Fragile X syndrome: A review of clinical management. Intractable Rare

Dis Res. 2016;5(3):145-157. doi:10.5582/irdr.2016.01048

2. Dionne O, and Corbin F. An "omic" overview of fragile X syndrome. Biology (Basel). 2021;10(5). doi:10.3390/biology10050433

3. Mila M, Alvarez-Mora MI, Madrigal I,Rodriguez-Revenga L. Fragile X syndrome: An overview and update of the FMR1 gene. Clin Genet. 2018;93(2):197-205. doi:10.1111/cge.13075

4. Kraan CM, Godler DE, Amor DJ. Epigenetics of fragile X syndrome and fragile X-related disorders. Dev Med Child Neurol. 2019;61(2). doi:10.1111/dmcn.13985

5. Nolin SL, Glicksman A,Tortora N, et al. Expansions and contractions of the FMR1 CGG repeat in 5,508 transmissions of normal, intermediate, and premutation alleles. Am J Med Genet Part A. 2019;179(7). doi:10.1002/ajmg.a.61165

6. Saldarriaga W, Tassone F, González-Teshima LY, Forero-Forero JV, Ayala-Zapata S,Hagerman R. Fragile X Syndrome. Colomb Med. Published online 2014. doi:10.25100/cm.v45i4.1810

7. Bagni C, Tassone F, Neri G,Hagerman R. Fragile X syndrome: Causes, diagnosis, mechanisms, and therapeutics. J Clin Invest. 2012;122(12). doi:10.1172/JCI63141

8. Terracciano A, Chiurazzi P,Neri G. Fragile X syndrome. Am J Med Genet - Semin Med Genet. 2005;137 C(1). doi:10.1002/ajmg.c.30062

9. Ferreira MES, de Vasconcelos AS,da Costa Vilhena T, et al. Oxidative Stress in Alzheimer's Disease: Should We Keep Trying Antioxidant Therapies? Cell Mol Neurobiol. 2015;35(5). doi:10.1007/s10571-015-0157-y

10. Tholey G, and Ledig M. Neuronal and astrocytic plasticity: Metabolic aspects. In: Annales de Medecine Interne. Vol 141. Suppl:138 ; 1990.

11. El Bekay R, Romero-Zerbo Y,Decara J, et al. Enhanced markers of oxidative stress, altered antioxidants and NADPH-oxidase activation in brains from Fragile X mental retardation 1-deficient mice, a pathological model for Fragile X syndrome. Eur J Neurosci. 2007;26(11). doi:10.1111/j.1460-9568.2007.05939.x

12. Ryter SW, Hong PK, Hoetzel A, et al. Mechanisms of cell death in oxidative stress. Antioxidants Redox Signal. 2007;9(1). doi:10.1089/ars.2007.9.49

13. Ghosh N, Das A, Chaffee S, Roy S,Sen CK. Reactive oxygen species, oxidative damage and cell death. In: Immunity and Inflammation in Health and Disease: Emerging Roles of Nutraceuticals and Functional Foods in Immune Support. ; 2017. doi:10.1016/B978-0-12-805417-8.00004-4

14. Birben E, Sahiner UM, Sackesen C, Erzurum S,Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1). doi:10.1097/WOX.0b013e3182439613

15. Venditti P, Di Stefano L, Di Meo S. Mitochondrial metabolism of reactive oxygen species. Mitochondrion. 2013;13(2). doi:10.1016/j.mito.2013.01.008

16. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1). doi:10.1016/j.biocel.2006.07.001

17. Mirończuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. Adv Med Sci. 2018;63(1). doi:10.1016/j.advms.2017.05.005

18. Rahal A, Kumar A, Singh V, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. Biomed Res Int. 2014;2014. doi:10.1155/2014/761264

19. Yasui K,Baba A. Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. Inflamm Res. 2006;55(9). doi:10.1007/s00011-006-5195-y

20. Nandi A, Yan LJ, Jana CK, Das N. Role of Catalase in Oxidative Stress- And Age-Associated Degenerative Diseases. Oxid Med Cell Longev. 2019;2019. doi:10.1155/2019/9613090

21. Lu SC. Regulation of glutathione synthesis. Mol Aspects Med. 2009;30(1-2). doi:10.1016/j.mam.2008.05.005

22. Torres LL, Quaglio NB, De Souza GT, et al. Peripheral oxidative stress biomarkers in mild cognitive impairment and alzheimer's disease. J Alzheimer's Dis. 2011;26(1). doi:10.3233/JAD-2011-110284

23. Kim GH, Kim JE, Rhie SJ, Yoon S. The Role of Oxidative Stress in Neurodegenerative Diseases. Exp Neurobiol. 2015;24(4). doi:10.5607/en.2015.24.4.325

24. Gandhi S, Abramov AY. Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev. Published online 2012. doi:10.1155/2012/428010

25. Dasuri K, Zhang L, Keller JN. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. Free Radic Biol Med. 2013;62. doi:10.1016/j.freeradbiomed.2012.09.016

26. Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. Biochim Biophys Acta - Gen Subj. 2013;1830(5). doi:10.1016/j.bbagen.2012.11.020

27. Power JHT, Blumbergs PC. Cellular glutathione peroxidase in human brain: Cellular distribution, and its potential role in the degradation of Lewy bodies in Parkinson's disease and dementia with Lewy bodies. Acta Neuropathol. 2009;117(1). doi:10.1007/s00401-008-0438-3

28. Brigelius-Flohé R, Flohé L. Regulatory Phenomena in the Glutathione Peroxidase Superfamily. Antioxidants Redox Signal. 2020;33(7). doi:10.1089/ars.2019.7905

29. Saluto A, Brussino A, Tassone F, et al. An enhanced polymerase chain reaction assay to detect pre- and full mutation alleles of the fragile X mental retardation 1 gene. J Mol Diagnostics. 2005;7(5). doi:10.1016/S1525-1578(10)60594-6

30. Zafarullah M, Tassone F. Molecular biomarkers in fragile x syndrome. Brain Sci. Published online 2019. doi:10.3390/brainsci9050096

31. Kumari D, Somma V, Nakamura AJ, Bonner WM, D'Ambrosio E, Usdin K. The role of DNA damage response pathways in chromosome fragility in Fragile X syndrome. Nucleic Acids Res. 2009;37(13). doi:10.1093/nar/gkp391

32. Lovell MA, Ehmann WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in alzheimer's disease. Neurology. 1995;45(8). doi:10.1212/WNL.45.8.1594

33. McCaddon A, Hudson P, Hill D, et al. Alzheimer's disease and total plasma aminothiols. Biol Psychiatry. 2003;53(3). doi:10.1016/S0006-3223(02)01451-8

34. Oakley AE, Collingwood JF, Dobson J, et al. Individual dopaminergic neurons show raised iron levels in Parkinson disease. Neurology. 2007;68(21). doi:10.1212/01.wnl.0000262033.01945.9a

35. Argüelles S, Machado MJ, Ayala A, Machado A, Hervías B. Correlation between circulating biomarkers of oxidative stress of maternal and umbilical cord blood at birth. Free Radic Res. 2006;40(6). doi:10.1080/10715760500519834

36. Dalvi SM, Patil VW, Ramraje NN. The roles of glutathione, glutathione peroxidase, glutathione reductase and the carbonyl protein in pulmonary and extra pulmonary tuberculosis. *J Clin Diagnostic Res*. 2012;6(9). doi:10.7860/JCDR/2012/4410.2533

37. Kosmachevskaya O V., Novikova NN, Topunov AF. Carbonyl stress in red blood cells and hemoglobin. *Antioxidants*. 2021;10(2). doi:10.3390/antiox10020253

38. Lovell MA, Ehmann WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in alzheimer's disease. *Neurology*. 1995;45(8). doi:10.1212/WNL.45.8.1594