

Oxidative Stress in Attention Deficit Hyperactivity Disorder Patients

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ABSTRACT

Background: Attention Deficit Hyperactivity Disorder (ADHD) is a mental disorder diagnosed by the symptoms of inattention, hyperactivity and impulsivity when the extent of symptoms impairs the child's ability to function. The underlying pathaetiological basis of ADHD remains unknown. Oxidative stress resulting in cell damage, mainly through the production of reactive oxygen species (ROS), has been implicated in various psychiatric disorders. Byproducts of lipid peroxidation as Malondialdehyde (MDA), transition metals as zinc, and levels of Non-Estrified Fatty Acids (NEFA) can be used as indirect biochemical indicators of oxidative stress. **Objective:** To elucidate the possible role of oxidative stress in ADHD patients compared with normal controls we assessed different biochemical markers of disrupted oxidative metabolism. **Method:** We recruited 20 children diagnosed with ADHD using DSM-IV criteria and 16 matched healthy controls. Both were subjected to the Wisconsin Card Sorting Test and patients were further categorized by Conner's Parent Rating Scale-Revised. Levels of MAD, Zinc, and NEFA were assessed in both groups. **Results:** There was evidence of increased lipid oxidation with evidence of disturbed lipid peroxidation. The increased MAD level compared with control (94.1 ± 32.2 , 39 ± 21.9 $p < 0.001$) was correlated to attention/hyperactivity predominantly inattentive. Additionally zinc levels were decreased compared to control (69 ± 16.9 , 95.5 ± 8.8 $p < 0.001$); but the difference was not statistically correlated with any ADHD subtypes. **Conclusion:** the increased MAD level as a lipid peroxidation marker and the decreased zinc status and their correlation with the inattention profile are in support of the oxidative stress theory in the pathogenesis of ADHD. This may point to the potential implication of antioxidants as a treatment option for ADHD.

Key words: Oxidative stress, Attention Deficit Hyperactivity Disorder, Antioxidants, Malonaldehyde.

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INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is one of the overt behavioural problems that starts as early as three years of age and peaks at school entrance. It is primarily characterised by a "persistent

pattern of inattention and/or hyperactivity-impulsivity that is more frequent and severe than is typically observed in individuals at a comparable level of development¹. Consequently, children with ADHD will

often experience serious problems functioning at the school and home environment which can disrupt their life, families, and friendships.

The American Psychiatric Association (1994), estimates that 3-5% of school aged children have ADHD, while other sources report higher prevalence rates ranging from 5-13%²⁻³. It is now recognised in the Arab world with an increasing pace being the “diagnosis of the decade” over the past ten years. Despite the intensive current ground research regarding the theories of ADHD it is still conflicting⁴. There is yet no single aetiology has been identified for ADHD and findings are much more consistent with a “*multi-factorial hypothesis*”. Since a number of the physical and behavioural symptoms of essential fatty acid deficiency can mimic some of the symptoms described in typical ADHD patients. Therefore it is conceivable, that either dietary deficiency of omega-3 fatty acids, or altered metabolic handling of these fatty acids, could contribute to the abnormalities observed in those affected by ADHD. Several studies examined the concentrations of the major circulating non-esterified fatty acids (myristate, palmitate, oleate, stearate, and arachidate) status in patients with behavioural disorders⁵⁻⁶.

Yet, fewer studies evaluated the biochemical basis of ADHD from a wider syndromic perspective, as ADHD may be associated with several other biochemical systems, such as oxidative metabolism⁷.

Reactive oxygen species (ROS), by-product of oxygen metabolism, can be evaluated indirectly by measuring some antioxidant enzyme levels such as superoxide dismutase, catalase or glutathione peroxidase, by-products of lipid peroxidation such as malondialdehyde

(MDA), or by some transition metal levels such as copper, zinc and iron^{3,8}.

Lipid peroxidation is a well-established mechanism of cellular injury in humans, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids (PUFA), are unstable and decompose to form a complex series of compounds⁹⁻¹⁰. These include reactive carbonyl compounds, and one of the most abundant and destructive is the intracellular aldehyde, malondialdehyde (MDA) a breakdown product of the major chain reaction of oxidation of polyunsaturated fatty acid (PUFA)¹¹. Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation^{7,12}.

Recent studies demonstrated that oxidative stress may have a role in the pathogenesis of various psychiatric disorders. Additionally, research groups have reported elevated MDA activity in various psychiatric diseases¹³⁻¹⁶. These findings suggest that increased MDA, a destructive agent, could have an important role in the pathophysiology of psychiatric diseases⁸.

This work was designed to elucidate the possible role of oxidative stress in ADHD patients compared with normal controls by assessing different biochemical markers of disrupted oxidative metabolism through evaluating by-products of lipid peroxidation as; MDA, antioxidant transition metals as zinc, and levels of Non-Esterified Fatty Acids (NEFA) aiming to explore their putative role in the pathogenesis of ADHD and its core symptomatology.

SUBJECTS AND METHODS

After obtaining the approval of the local Ethical Committee at the Institute of Psychiatry, Ain Shams University, each parent or caregiver gave an informed consent prior to participation in the study. We were able to recruit 20 medication naïve children from the ADHD Psychiatric Outpatient Clinic, Ain Shams University Hospitals, Cairo, Egypt diagnosed with ADHD according to the DSM-IV criteria. The sample consisted of 17 males (85%) and 3 females (15%) with an age range of 7 to 15 years (mean=8.9±2.5 years). They were compared to 16 healthy controls (13 males, 81%; 3 females, 19%) from visitors of the paediatrics department matched for age, educational and social level. The mean age of control group was 9.0±2.2 (range 7-15 years). Each participant was subjected to a multidisciplinary clinical interview using a comprehensively devised semi-structured sheet covering sociodemographics, psychiatric and paediatric history and physical examination. Both patients and control group were subjected to Wisconsin Card Sorting Test¹⁷.

Twelve hours fasting blood was collected by venipuncture into EDTA vacutainer tubes. Centrifugation was done at 1300xg for 10 minutes and separated plasma was stored immediately following centrifugation at -70°C. The ADHD patients' group were further subtyped according to DSM-IV into; Attention Deficit/Hyperactivity disorder, combined type (N=8), Predominantly Inattentive type (N=7), Predominantly Hyperactive-Impulsive type (N=5). Additionally, the patients were categorised based on Conners' Rating Scale-Revised (CPRS: L)¹⁸ (table 1).

Description of tools:

1- Conners' rating scales-revised long form (CPRS- R -L): A multidimensional scale

used to measure ADHD and to recognise the deficits related to the disorder. It is linked to DSM-IV-R criteria and covers a variety of settings (home-School). It has an excellent reliability and validity with internal consistency coefficient range 0.75-0.90, and test retest reliability range from 0.60-0.90. The scale applied was the Arabic adapted rendition of the Conners' Parent Rating Scale Revised-Long version translated, culturally adapted, and validated¹⁹. The scale consist of an 80 item questionnaire with an average administration time is 25-30 min. Scoring items of the parent's report on the child's behaviour during the past month on 14 subscales; oppositional, cognitive problems and inattention, hyperactivity, anxious, shy, perfectionism, social problems, and psychosomatic.

Table (1): Clinical characteristics of patients

	Variables	N	%
Sex	Male	17	85
	Female	3	15
ADHD subtypes	ADHD / combined	8	40%
	ADHD / inattentive	7	35%
	ADHD/ hyperactive-impulsive	5	25%
Conner's Scale	Hyperactivity	13	65
	Anxious	3	15
	Social problem	3	15
	Perfection	0	0
	Liability	7	35
	Inattention	14	70
	Hyperactivity/ Impulsive	13	65

2- Wisconsin Card Sorting Test: The WCST assesses abstract reasoning ability and the ability to shift cognitive strategies in response to changing environmental contingencies¹⁷. The test can be considered a measure of executive function requiring the ability to develop and maintain an appropriate problem solving strategy across changing stimulus conditions in order to

achieve a future goal. It requires strategic planning, organized searching, utilising environmental feedback to shift cognitive sets, directing behaviour toward achieving goals and modulating impulsive responses²⁰⁻²¹. WCST provides scores not only of overall success, but also specific sources of difficulty in the task (e.g. inefficient initial conceptualisation, failure to maintain cognitive set, perseveration and inefficient learning across stages of the test. The WCST consists of four stimulus cards and 128 response cards that depict figures of varying forms (crosses, circles, triangles, or stars), colours (red, blue, yellow, or green) and numbers of figures (one, two, three, or four). With the computerised WCST, the examiner must instruct the subject to make response selections using the keyboard keys. The first correct sorting strategy is colour, the process continues until the subject has produced 10 consecutive colour responses. Without comment or any indication the correct sorting category is changed to form which remains again until 10 consecutive correct responses are attained and subsequently the correct sorting category to number. After 10 consecutive correct responses to number it will switch back to colour as the correct sorting category and then to form and then to number in the manner as described. The test continues until the subject has successfully completed 6 categories or until all responses could have been used, whichever comes first. Regarding scoring, each response the subject makes can be thought of as occurring on three separate dimensions, correct or incorrect, ambiguous or unambiguous, and perseverative or nonperseverative. According to the above mentioned data, selected scoring area includes categories completed, failure to maintain set and conceptual level response.

WCST demonstrates sensitivity and specificity for the identification of those executive function deficits associated with ADHD²².

Laboratory testing:

MDA Assay: The plasma MDA levels were determined based on the reaction of MDA with Thiobarbituric acid (TBA) at 95°C²³. In the TBA test reaction, MDA and TBA react to form a pink colour with an absorption maximum at 532nm. The reaction was performed at pH 2–3 at 95°C for 15 minutes. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was allowed to react with an equal volume of 0.67% TBA in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 532nm. Arbitrary values obtained were compared with a series of standard solutions (1, 1, 3, 3 tetramethoxypropane). Results were expressed as nmol/mL.

Zinc Assay: Quantitative Colorimetric Zinc Determination was done. This method utilises a chromogen that forms a coloured complex specifically with zinc. 200uL water, standard, sample and sample blank (200uL+ 8uL EDTA) were transferred to appropriately labelled tubes. 800uL working reagent was added and taped lightly to mix. Incubated 30 min and read optical density at 425nm. The intensity of the colour, measured at 425nm, is directly proportional to the zinc concentration in the sample. Zinc is expressed as µg/dL.

Non Estrified Fatty Acids Assay (NEFA): NEFA were assayed using the Wako enzymatic method²⁴. It relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA

synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide, in the presence of peroxidase (POD) permits the oxidative condensation of 3-methy-N-ethyl-N(β -hydroxyethyl)-aniline (MEFA) with 4-aminoantipyrine to form a purple colour which can be measured colorimetrically at 550nm. It expressed as mEq/L²⁵.

Statistical analysis:

Data analysis was done using Statistical Package for Social Sciences Version-10 (SPSS-10). *Student's T Test (t)* was used for comparison between means of the different groups of patients. *Pearson Chi-Square Test (χ^2)* was used for comparison between qualitative variables. *P value* was used to indicate the level of significance ($P \leq 0.05$ is considered significant, $P \leq 0.01$ is highly significant, $P \leq 0.001$ is very highly significant). Kruskal and Wallis test was

used to estimate the non-parametric correlation²⁶.

RESULTS

We found MDA levels showed statistically significant ($p=0.030$) association with the diagnostic subtype of ADHD, while the levels of zinc and NEFA did not reach a statistical significance (table 2). The ADHD group had significantly higher MDA ($p<0.00$) and NEFA ($p<0.007$) levels (table 3), and significantly lower levels of zinc ($p<0.00$). The mean serum NEFA level in the patient group was 572.7 ± 177.4 mEq/L and in control group, 377.6 ± 236.1 mEq/L. The mean serum zinc level of patient group was 69 ± 16.9 micrograms/dl and that of the control group, 95.5 ± 8.8 micrograms/dl. The mean serum MDA level of patient group was 94.1 ± 32.3 micrograms/dl and that of the control group, 39 ± 21.9 micrograms/dl.

Table (2): Correlation between laboratory indices and subtypes of ADHD

Varia-ble	Subtypes means $\pm$ SD	Kuskall Wallis	
		Chi Square	P
NEFA	ADHD / combined $662.25 \pm$ SD	3.49	0.17
	ADHD / inattention 516.5 ± 103.3		
	ADHD/ Hyperactive 508 ± 192.8		
Zinc Level	ADHD / combined 175 ± 17.1	3.800	0.150
	ADHD / inattention 59.8 ± 13.7		
	ADHD/ Hyperactive 72.0 ± 18.23		
MDA	ADHD / combined 70.6 ± 35.8	6.473*	0.030
	ADHD / inattention 112.0 ± 4.9		
	ADHD/ Hyperactive 106.8 ± 28.4		

Table (3): Laboratory levels and Wisconsin categories in patients and control

Variable	Patient Mean $\pm$ SD	Control Mean $\pm$ SD	Mann-Whitney U test	P
NEFA	572.7 ± 177.4	377.6 ± 236.1	75.0	0.007
Zinc level	69 ± 16.9	95.5 ± 8.8	25.0	0.000
MDA	94.1 ± 32.3	39 ± 21.9	32.00	0.000
Wisconsin % conceptual level	33.1 ± 14.3	78.3 ± 5.5	7.00	0.001
Wisconsin category completed	2.7 ± 1.08	6.7 ± 1.09	3.00	0.000
Wisconsin failure to maintain a set	0.25 ± 0.7	0.5 ± 0.0	86.5	0.184

Table (4): Showing a highly statistical correlation between MDA, Zinc levels and Wisconsin % and category completed.

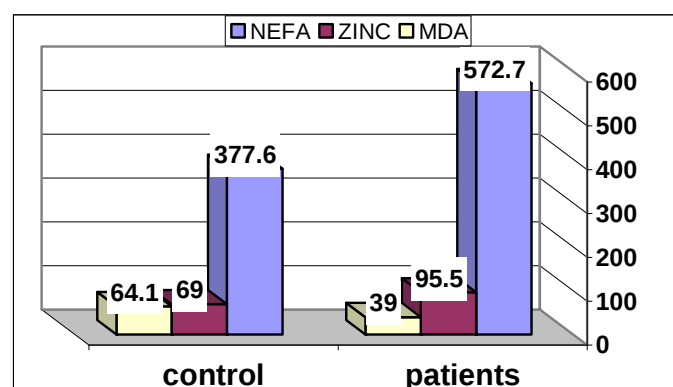
Variable	MDA	Zinc level	Non esterifies fatty acid	Wisconsin % conceptual level	Wisconsin category completed	Wisconsin failure to maintain a set
Wisconsin % conceptual level						
Correlation coefficient	-0.511**	0.619**	-0.248	1.000	0.771**	0.137
P value	0.003	0.000	0.178	-	0.000	0.461
Wisconsin category completed						
Correlation coefficient	-0.535**	0.552**	-0.448**	0.771	1.000	0.132
P value	0.002	0.001	0.000	0.000	-	0.479
Wisconsin failure to maintain a set						
Correlation coefficient	-0.012	0.140	-0.083	0.137	0.132	1.000
P value	0.948	0.453	0.658	0.479	0.479	-

** Highly significant; *Significant

Table (5): Showing a highly statistical correlation between MDA and Zinc levels and Wisconsin % and category completed.

Variable	MDA	Zinc level	NEFA	Wisconsin % conceptual level	Wisconsin category completed	Wisconsin failure to maintain a set
MDA						
Correlation coefficient	1.000	-0.609**	0.244	-0.511**	-0.535	-0.012
P value	-	0.000	0.157	0.003	0.002	0.948
Zinc level scores						
Correlation coefficient	-0.609	1.000	-0.026	0.619**	0.552**	0.140
P value	0.000	-	0.881	0.000	0.001	0.453
NEFA						
Correlation coefficient	0.244	0.026	1.000	-0.248	-0.448**	-0.083
P value	0.157	0.881	-	0.178	0.012	0.658

** Highly significant; * Significant

**Fig. (1): Comparison between levels of NEFA, zinc and MDA in ADHD patients and control group.**

DISCUSSION

There is rising evidence that in ADHD oxidative stress leads to an imbalance between the production of reactive oxygen species (ROS), and the biological system's ability to detoxify the reactive intermediates. This ability to maintain a reducing environment within the cells requires constant input of metabolic energy²⁷. Any disturbances in the normal redox state will cause toxic effects through the production of compounds as peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA²⁸. Oxidative lipid degeneration and its by-products may play an important role in cell dysfunction, and hence that can be fundamental to aetiopathogenesis of ADHD⁸. Our findings above indicate that there is deranged oxidative lipid metabolism and reduction of antioxidant trace elements suggestive of an underlying oxidative stress in ADHD.

The aetiology of ADHD is thought to include abnormal regulation of the neurotransmitter system particularly dopamine and norepinephrine²⁹⁻³⁰. The oxidative stress can disrupt the synaptic cell membrane affecting neurotransmitter function⁸. Dysfunction in the membrane transporter system cause accumulation of Catecholamines in synaptic cleft where they are subjected to enzymatic oxidation by monoamine oxidase (MAO) or oxygen mediated non-enzymatic degradation. Both generating more ROS and increasing the free radical burden leading to more oxidative damage to cell membrane³¹⁻³². Attempts to disrupt this vicious circle by antioxidant treatment may be a potential therapeutic target.

ADHD and malondialdehyde level:

Numerous studies indicated that free radical-mediated neuronal damage plays a role in the pathophysiology of various psychiatric disorders¹³. Higher MDA levels may be indicative of the underlying pathophysiology in ADHD. A remarkable increase in MDA levels suggests a strong association between ADHD and lipid oxidation defect. Our study showed increased significantly higher mean plasma MDA in ADHD patients compared to controls (94.1 ± 32.3 , 39 ± 21.9 $p < 0.001$). The same results were found in Adult ADHD. A study reported increased MDA levels in A-ADHD⁸. Also in a recent Egyptian study of 21 A-ADHD subjects and 20 healthy controls to investigate the role of free radicals and antioxidants by measuring plasma malondialdehyde, vitamin C, vitamin E concentrations and erythrocyte glutathione peroxidase; Eissa reported significantly higher plasma levels of malondialdehyde in A-ADHD patients³³. These findings could be interpreted as an indication of an increased oxidative stress in adult version of this disorder. Hence, oxidative stress may possibly be a trait in ADHD that plays a continuous pathological role across its course or even mediate a state that is conducive to some of its symptoms.

We found that lipid peroxidation as indicated by increased MDA levels was statistically correlated to ADHD/Inattentive subtype in children. Hyperactivity typically wanes as children get older, while attention and organizational problems can persist into adulthood³⁴. Our finding, along with adult ADHD data indicating increased MDA, and

the fact that inattention persists into adulthood may suggest that MDA is a possible potential marker for inattention in ADHD. Such hypothesis needs to be tested in comparative studies of child and adult ADHD.

Zinc level and ADHD: A number of studies pointed to an association between zinc deficiency and ADHD. Our results showed a highly significant decrease in zinc in children with ADHD than controls (69 ± 16.9 , 95.5 ± 8.8 , $p < 0.001$). The same result was reported (35) in ADHD patients who had significant ($p < 0.001$) reduction in mean zinc levels compared to controls (60.6 ± 9.9 vs. 105.8 ± 13.2 micrograms/dl). The level of zinc deficiency in the ADHD group in this study showed high statistical positive correlation with the decrease in mean FFA levels suggesting a relationship between zinc and FFA. Whether this is an epiphenomenon due to zinc deficiency or a true association is still to be investigated. Nevertheless in our study zinc levels did not show any statistical correlation with NEFA levels which were high; but a statistical negative correlation with the lipid degeneration by-product (MDA). Our finding implies that zinc deficiency may cause increase in oxidative stress. Some studies additionally indicated that it can possibly decrease antioxidant defense system as well³⁶⁻³⁸.

Along its effects on oxidative system, zinc deficiency may lead to neurotransmitter dysfunction. Zinc is an important co-factor for metabolism relevant to neurotransmitters affecting melatonin and dopamine metabolism. Melatonin has a direct action on serotonin, nor-epinephrine, and dopamine³⁹. The latter believed to be intimately associated with ADHD⁴⁰⁻⁴¹. Dopamine transporter (DAT) plays a role in

dopamine metabolism. Most of the studies showed a higher DAT availability in untreated patients with ADHD compared with controls⁴². Zinc is a DAT inhibitor and hence may indirectly affect dopamine metabolism⁴³. This disruption may have implication on symptomatology as well as treatment of ADHD.

Our results revealed that the ADHD group, who were zinc deficient compared to controls, had a statistically significant scoring difference on WCST indicating executive function defect and poor information processing. Moreover, there was significant positive correlation between zinc level and Wisconsin percent conceptual level and Wisconsin categories completed; but not failure to maintain set. These results are reflective of the finding that examined the relationship between plasma zinc levels and event-related potentials (ERPs) indices using an auditory oddball paradigm in ADHD patients and controls⁴⁴. Results highlighted a medium but significant positive correlation between plasma zinc levels and amplitude and latency of frontal N2 and P3 waves in ADHD. These results suggest that plasma zinc levels may have an effect on information processing in ADHD children, and that lower zinc levels seem to affect N2 wave which may reflect a possible different inhibition process in children with zinc-deficient ADHD.

The putative role of Zinc on ADHD core symptoms is further elucidated by its positive therapeutic effect in a double-blind, placebo-controlled study showing that Zinc sulphate was statistically superior to placebo in reducing both hyperactive, impulsive and impaired socialization symptoms, but not in reducing attention deficiency symptoms⁴⁵. Also it was noted

that: "behaviour much improved in children taking zinc, yet this group of patients were three-times more likely to complain of nausea and the metallic taste of the supplement"⁴⁶.

Furthermore, zinc supplement can enhance stimulant treatment effectiveness. In a balanced crossover design study to explore the relationship between zinc nutrition and stimulant therapy found that some ADHD children may be mildly deficient in zinc and constitute poorer stimulant responders⁴⁷. In a further analysis ADHD subjects treated by d-amphetamine were categorized as zinc-adequate, borderline zinc, and zinc-deficient by hair, red cell, and urine zinc levels⁴⁰. Results showed that placebo-controlled d-amphetamine treatment response appeared linear with zinc nutrition. Another controlled trial assessed the benefits of prescribing supplementary zinc alongside methylphenidate treatment found that children taking additional zinc sulphate on a daily basis improved faster than those taking a placebo. Some researchers even suggested that supplements may be of equal efficacy to other stimulant therapy⁴⁸. Hence, evidence seems to support the possible role of zinc deficiency in the pathogenesis of ADHD⁴⁶.

ADHD and free fatty acid level: Evidence suggests that elevated free fatty acids (FFA) may enhance oxidative stress⁴⁹. FFAs adversely affect mitochondrial function, resulting in increased in the production of reactive oxygen species⁵⁰. In our study, the mean serum NEFA level in the patient group was 572.7 ± 177.4 mEq/L and in control group was 377.6 ± 236.1 mEq/L ($p < 0.001$) with no statistical correlation to zinc levels. These results conflict with the finding that children with ADHD and decreased zinc levels have lower

proportions of FFA where the mean FFA level in ADHD group was about one-third of that of the control group ($p < 0.001$)³⁵. There was no correlation between zinc and FFA levels found in control group, while a statically significant correlation was found in ADHD group.

It is therefore important for further NEFA in ADHD by measuring and controlling for the various confounding variables that can affect serum FFA composition including; dietary fat intake, weight, gender, serum zinc, and EFA levels⁵¹. Patients with ADHD may have increased incidence of abnormal eating behaviour and bingeing⁵² which can alter serum FFA levels and cause increase in weight⁵³. Obesity can result in elevated total NEFA concentrations⁶. Gender as well an important determinant, Rogiers found a statistical significant gender difference in NEFA, where monounsaturated fatty acids were significantly higher in boys compared to girls⁵⁴. Zinc levels can also influence serum FFA levels and composition as it is a constitutive cofactor of Δ -desaturase enzyme activity essential for endogenous fatty acid synthesis⁵¹. Essential fatty acids (EFA) modulate plasma lipids, and deficiency in animal models can cause increase in FFA⁵⁵⁻⁵⁶. Moreover, studies should investigate differential serum FFA composition using techniques as gas chromatography mass spectrometry, as the colorimetric assays detect only the total concentrations of NEFAs, they give no information on the relative amounts of saturated and unsaturated fatty acids⁶.

ADHD, essential fatty acids, and oxidative stress: EFAs are not synthesized in the body; however EFAs and their products are necessary for maintenance of the structural and functional integrity of neurons and are

crucial for brain development⁵⁷⁻⁵⁸. Animal Models deficient in EFAs exhibit behavioural, sensory, and neurological dysfunction⁵⁹⁻⁶¹. In humans, lower blood levels of EFAs are associated with a variety of manifestations⁶². Deficiency of EFAs may lead to impaired growth, dry and scaly skin, polydipsia, and polyuria, and other somatic symptoms^{4-5, 63}. Moreover, children with lower EFAs levels display more behavioural problems, and temper tantrums as well as learning, health, and sleep problems⁶⁴. Another study reported similar symptoms that mimic fatty acid deficiency symptoms (FADS) in a subgroup of ADHD patients with low EFA levels and suggested possible contributing factors as; marginal consumption of EFA, or altered metabolic handling through inefficient conversion of EFA into Long Chain Polyunsaturated Fatty Acid (LCPUFA) and/or enhanced metabolism of LCPUFAs⁴. Colter and colleagues reported that ADHD children consumed equivalent amounts of omega-3 and omega-6 fatty acids to controls, however they had significantly lower levels of docosahexaenoic acid (DHA, 22:6n-3) and total omega-3 fatty acids. Hence, altered EFA metabolism appears to be a plausible hypothesis explaining the underlying abnormal essential fatty acid profiles that are often observed in ADHD⁶⁵. This metabolic abnormality may play a crucial role affecting the oxidant/antioxidant status in individuals with ADHD, and alternatively the potential of oxidative tissue damage. Some biochemical research suggested that essential fatty acid (EFA), especially omega-3, can reduce various oxidant parameters and enhance resistance to free radical attack and reduce lipid peroxidation⁶⁶⁻⁶⁸. On the other hand excess prolonged intake of PUFA increase the

incorporation of (n-3) fatty acids, mainly DHA which is readily oxidized, into plasma and tissue lipids may undesirably enhance susceptibility of membranes to lipid peroxidation and disrupt the antioxidant system⁶⁹.

Therefore, while preliminary evidences suggest that EFA supplementation might help in the management of the ADHD linked attentional, behavioural, cognitive and learning difficulties^{65, 70-71}, at least in a subpopulation of children and adolescents with ADHD⁷²⁻⁷³, it is prudent that this intervention should be coupled with other antioxidant therapy to minimize the potential peroxidative damage of tissue lipids due to excess accumulation of DHA. Furthermore, research should endeavor to investigate reliable clinical and biochemical selection criterion for individuals with ADHD who may benefit from EPA supplementation, and whether fatty acid deficiency symptoms (FADS) per se is an indication of such an intervention.

CONCLUSION

The aetiology of ADHD is complex and multi-factorial. Fatty acids have fundamental structural and functional roles in the central nervous system. At least some features of ADHD may reflect an underlying abnormality of fatty acid metabolism. The increased malonaldehyde level as a lipid peroxidation marker and the decreased zinc status and their correlation with the inattention profile provide evidence for the oxidative stress theory in the pathogenesis of ADHD, and hence the possible implication of antioxidants as a new line of treatment. Our findings may provide a scientific guide for the further clinical and biochemical studies on this

disorder. Therefore the need of larger scale studies especially those exploring the levels of other non-enzymatic and enzymatic antioxidants to verify the possible association between oxidative stress and ADHD and elucidate further potential treatment interventions.

Abbreviations used: A-ADHD, Adult Attention-Deficit/Hyperactivity Disorder; ACOD, acyl-CoA oxidase; ACS, Acyl-CoA synthetase; ADHD, Attention-Deficit/Hyperactivity Disorder; CPRS: L, Conners' Rating Scale-Revised; DHA, docosahexaenoic acid; EDTA, ethylenediaminetetraacetic acid; ERPs, Event-Related Potentials; FFA, Free Fatty Acids; LCPUFA, Long Chain Polyunsaturated Fatty Acid; MDA, malondialdehyde; MEFA, 3-methyl-N-ethyl-N (β -hydroxyethyl)-aniline; NEFA, Non-Esterified Fatty Acids; POD, peroxidase; PUFA, polyunsaturated fatty acid; ROS, Reactive Oxygen Species; WCST, Wisconsin Card Sorting Test.

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