

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. As with many psychiatric disorders, 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. Many findings suggest that lipid peroxidation, in particular, may play a central role in the pathophysiology of different mental disorders including ADHD. **Aim of the work:** To elucidate the possible role of oxidative stress in ADHD patients compared with normal controls through assessing different biochemical markers of disrupted oxidative metabolism. **Methods:** The study recruited twenty children diagnosed with ADHD using DSM-IV criteria and sixteen matched healthy controls. Both were subjected to comprehensive psychiatric evaluation and the Wisconsin Card Sorting Test to assess cognitive set shifting, planning and conceptualisation. Patients with ADHD were further categorized by Conner's Parent Rating Scale- Revised to recognise the predominant deficit associated with the disorder. Levels of Malondialdehyde (MDA), Zinc, and Non-Estrified Fatty Acids (NEFA) were assessed in both groups. Laboratorial assay for Malonaldehyde was done using spectrophotometric method, while Non Esteified Fatty Acid and Zinc Levels were measured colorimetrically. **Results:** The current study revealed evidence of increased lipid oxidation with evidence of disturbed lipid peroxidation. The increased malonaldehyde 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. In conclusion, the increased malonaldehyde 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. Larger scale studies and additional research examining the levels of other non-enzymatic antioxidants as well as the enzymatic antioxidants in children with ADHD are required to verify this possible association.

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 development" (**Barkley, 1990**). 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% (**Hoza et al., 2001**). 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 conflicting (**Burgess et al., 2000**). 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 disorders (**Mitchell et al., 1987; Sabin et al., 2007**).

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 metabolism (**Varol et al., 2006**).

Reactive oxygen species (ROS), byproduct 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 (**Hoza et al., 2001; Bulut et al., 2007**).

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 compounds (**Marnett, 1999, 2002**). 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) (**Feng et al., 2006**). Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation (**Wascowicz et al., 1993; Varol et al., 2006**).

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 diseases (**Kuloglu et al., 2002; Ozcan et al., 2004; Ersan et al., 2006; Atmaca et al., 2008**). These findings suggest that increased MDA, a destructive agent, could have an important role in the pathophysiology of psychiatric diseases (**Bulut et al., 2007**).

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 Byproducts of lipid peroxidation as; Malondialdehyde (MDA), antioxidant transition metals as zinc, and levels of Non-Estrified Fatty Acids (NEFA) aiming to explore their putative role in the pathogenesis of ADHD and its core symptomatology.

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 Test (**Heaton, 1993**).

12 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) (Conners, 1997) as seen in (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 validated by **El Sheikh and colleagues (2002)**. 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.

2- Wisconsin Card Sorting Test (Heaton, 1993)

The Wisconsin Card Sorting Test (WCST) assesses abstract reasoning ability and the ability to shift cognitive strategies in response to changing environmental contingencies. 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 responses (*Cianchetti et al., 2007; Kremen et al., 2007*). 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. Wisconsin Card Sorting Test (WCST)

demonstrates sensitivity and specificity for the identification of those executive function deficits associated with ADHD (*Romine et al., 2004*).

3. Laboratory Testing:

Malondyaldehyde (MDA) Assay: The plasma MDA levels were determined by the method of Draper and Hadley (1990) based on the reaction of MDA with Thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form a pink colour with an absorption maximum at 532 nm. 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 532 nm. 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. 200 uL water, standard, sample and sample blank (200uL+ 8 u L EDTA) were transferred to appropriately labelled tubes. 800 uL working reagent was added and taped lightly to mix. Incubated 30 min and read optical density at 425 nm. The intensity of the colour, measured at 425 nm, is directly proportional to the zinc concentration in the sample. Zinc is expressed as µg/dL.

Non estrified fatty acids Assay: Non estrified fatty acids were assayed using the Wako enzymatic method (*Wako Diagnostics, 2006*). 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 550 nm. It expressed as mEq/L (**Jebens and Sejersted, 1992**).

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 (1952)** was used to estimate the non-parametric correlation.

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.

On the Wisconsin, the patients' group showed significantly lower mean percent conceptual level (33.1 ± 14.3) compared to control group, 78.3 ± 5.5 ($p < .001$), and lower mean category completion (2.7 ± 1.08 versus 6.7 ± 1.09 , $p < .000$).

But there was no statistical significance with regards mean Wisconsin failure to maintain a set ($p = 0.184$). We found high statistical correlation between MDA and Zinc; but not NEFA, and % conceptual level (Table 4). All three indices were highly statistically correlated to category completion and failed to show any significant statistically correlation with the failure to maintain a set.

Table (1): Clinical characteristics of patients group

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

Table (1) continue:

Anxious	3	15
Social problem	3	15
Perfection	0	0
Liability	7	35
Inattention	14	70
Hyperactivity / Impulsive	13	65

Table (2): showing the correlation between laboratory indices and subtypes of ADHD

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

Table (3) Showing 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 $\pm$ 177.4	337.6 $\pm$ 236.1	75.0	0.007
Zinc level	69 $\pm$ 16.9	95.5 $\pm$ 8.8	25.0	0.000
MDA	94.1 $\pm$ 32.3	39 $\pm$ 21.9	32.00	0.000
Wisconsin % conceptual level	33.1 $\pm$ 14.3	78.3 $\pm$ 5.5	7.00	0.001
Wisconsin category completed	2.7 $\pm$ 1.08	6.7 $\pm$ 1.09	3.00	0.000
Wisconsin failure to maintain a set	0.25 $\pm$ 0.7	0.5 $\pm$ 0.0	86.5	0.184

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

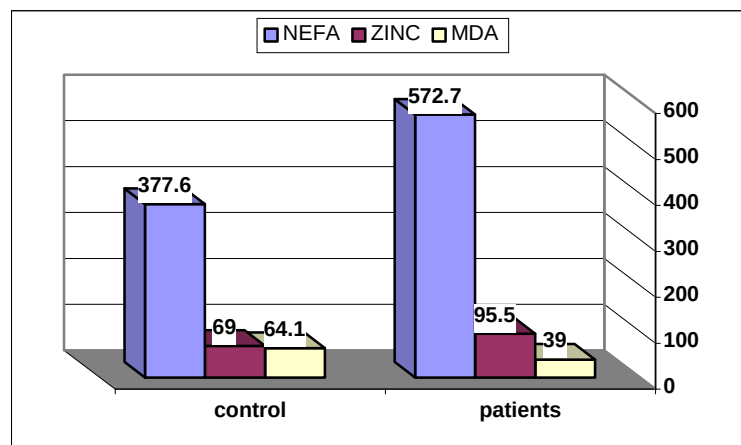
Variable	MDA	Zinc level	Non esterifies fatty acid	Wisconsin % conceptual level	Wisconsin category completed	Wisconsin failure to maintain a set
Wisconsin % conceptual level						
Correlation coefficient	-0.511**	0.619**	-0.248	1.000	0.771**	0.137
P value	0.003	0.000	0.178	-	0.000	0.461
Wisconsin category 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
Non esterifies fatty acid						
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): Showing 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 energy (Romero *et al.*, 1998). 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 DNA (Young, 2004). Oxidative lipid degeneration and its by-products may play an important role in cell dysfunction, and hence that can be fundamental to aetiopathogenesis of ADHD (Bulut, *et al.*, 2007). 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 norepinephrine (**Levy, 1991; Prince, 2008**). The oxidative stress can disrupt the synaptic cell membrane affecting neurotransmitter function (**Bulut, 2007**). 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 membrane (**Casarett, et al., 2001; Tezcan, et al., 2003**). 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 disorders (**Kuloglu et al., 2002**). 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 by Bulut and colleagues (2007) reported increased MDA levels in A-ADHD. 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,**

(2008) reported significantly higher plasma levels of malondialdehyde in A-ADHD patients. 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 adulthood (**Adler and Chua, 2002**). 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 by Bekaroğlu and colleagues, (1996) where ADHD patients 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. But; 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 well (**Duzguner and Kaya, 2007; Chen et al., 2007; Taysi et al., 2008**).

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 dopamine (**Benoit, 2007**). The latter believed to be intimately associated with attention-deficit/hyperactivity disorder (**Arnold et al., 2000; Faraone, 2005**). 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 controls (**Spencer et al., 2007**). Zinc is a DAT inhibitor and hence may indirectly affect dopamine metabolism (**Krause, 2008**). 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 of **Yorbik and colleagues (2008)** examining the relationship between plasma zinc levels and event-related potentials (ERPs) indices using an auditory oddball paradigm in ADHD patients and controls. 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. **Bilici and colleagues (2004)** in a double-blind, placebo-controlled study found that Zinc sulphate was statistically superior to placebo in reducing both hyperactive, impulsive and impaired socialization symptoms, but not in reducing attention deficiency symptoms. **Akhondzadeh and colleagues (2004)** 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".

Furthermore, zinc supplement can enhance stimulant treatment effectiveness. **Arnold and colleagues (1990)** 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 responders. In a further analysis (**Arnold et al., 2000**). ADHD subjects treated by d-amphetamine were categorized as zinc-adequate, borderline zinc, and zinc-deficient by hair,

red cell, and urine zinc levels. 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 therapy (**Harding, 2003**). Hence, evidence seems to support the possible role of zinc deficiency in the pathogenesis of ADHD (**Akhondzadel et al., 2004**).

ADHD and free fatty acid level

Evidence suggests that elevated free fatty acids (FFA) may enhance oxidative stress (**Paolisso et al., 1996**). FFAs adversely affect mitochondrial function, resulting in increased in the production of reactive oxygen species (**Santangelo et al., 2007**). 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 of **Bekaroglu and colleagues (1996)** that children with ADHD and decreased zinc levels have lower proportions of free fatty acids where the mean FFA level in ADHD group was about one-third of that of the control group ($p < 0.001$). 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 studies to investigate non-esterified free fatty acids 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 levels (**Wang et al., 2008**). Patients with ADHD may have increased incidence of abnormal eating behaviour and binging (**Cortese et al., 2007**) which can alter serum FFA levels and cause increase in weight (**Cortese et al., 2008**). Obesity can result in elevated total non-esterified fatty acid concentrations (**Sabin et al., 2007**). Gender as well an important determinant, **Rogiers (1981)** found a statistical significant gender difference in NEFA, where monounsaturated fatty acids were significantly higher in boys compared to girls. 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 synthesis (**Wang et al., 2008**). Essential fatty acids (EFA) modulate plasma lipids, and deficiency in animal models can cause increase in FFA (**Levy et al., 1990; Cherniavs'kyi et al., 2006**). 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 acids (**Sabin et al., 2007**).

ADHD, Essential Fatty Acids, and oxidative stress

Essential fatty acids (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 development (**Youdim et al., 2000; Singh, 2005**). Animal Models deficient in EFAs exhibit behavioural, sensory, and neurological dysfunction (**Enslen et al., 1991; Delion et al., 1994; Chalon et al.,**

1998). In humans, lower blood levels of EFAs are associated with a variety of manifestations (*Antalis et al., 2006*). Deficiency of EFAs may lead to impaired growth, dry and scaly skin, polydipsia, and polyuria, and other somatic symptoms (*Hansen et al., 1958; Mitchell et al., 1987; Burgess et al., 2000*). Moreover, children with lower EFAs levels display more behavioural problems, and temper tantrums as well as learning, health, and sleep problems (*Stevens et al., 1996*). **Burgess and colleagues (2000)** 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 LCPUFAs. **Colter and colleagues (2008)** 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 ADHD. 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 peroxidation (*Erdogan et al., 2004; Iraz et al., 2005; Bas et al., 2007*). 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 system (*Song et al., 2000*).

Therefore, while preliminary evidences suggest that EFA supplementation might help in the management of the ADHD linked attentional, behavioural, cognitive and learning difficulties (*Sorgi et al., 2007; Sinn and Bryan 2007; Colter et al., 2008*), at least in a subpopulation of children and adolescents with ADHD (*Sinn 2007; Johnson et al., 2008*), 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.

In 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.

Footnotes:

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-methy-N-ethyl-N (β -hydroxyethyl)-aniline; NEFA, Non-Estrified Fatty Acids; POD, peroxidase; PUFA, polyunsaturated fatty acid; ROS, Reactive Oxygen Species; WCST, Wisconsin Card Sorting Test.

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