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# Evaluation of plasma neurotransmitters in children living with Attention-Deficit Hyperactivity Disorder (ADHD)

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This study aimed to ascertain the underlying neuro-biochemical imbalances that exist in children with ADHD by assessing the plasma levels of dopamine, serotonin, norepinephrine, γ amino butyric acid (GABA) and glutamate. Moreover, it investigated the potential effects of PUFA and vitamins supplementation as an alternative therapy to modulate the levels of these neurotransmitters and the overall clinical status of ADHD patients. The study included 40 ADHD patients, aged 4-6 years. The diagnostic and statistical manual of mental disorders (DSM-5) test has been employed to diagnose patients with ADHD and the severity of symptoms was assessed using the Arabic version of Conners' Parent Rating Scale. Additionally, patients were assessed using the Arabic versions of Mini International Neuropsychiatric Interview for Children (M.I.N.I. Kid) and Stanford-Binet Intelligence Scale, 5th Edition (SB5). Recruited patient received nutritional supplement of semi-solid diet containing 1000 mg PUFA with selected vitamins once daily for six months. The evaluation of ADHD symptoms and levels of neurotransmitters has been carried out at pre-/post-intervention stage. Post-nutrition intervention assessment, there was a significant increase in dopamine, norepinephrine, GABA levels (p-value < 0.0001) with significant decrease in glutamate level (p-value < 0.0001) when compared to their correspondent pre-intervention levels. Symptoms of inattention and/or hyperactivity-impulsivity were significantly improved after 6 months nutrition intervention program (p-value<0.001). Therefore, supplementation with omega-3 fatty acids and vitamins could be considered more extensively in therapy of ADHD patients particularly those who are less than 6 years old

**Keywords: ADHD; Neurotransmitters; Dopamine; PUFA; Omega-3. semi-solid diet**

## INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a chronic neurodevelopmental disorder characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity; it begins in childhood, and affects cognitive abilities, personal, familial and social interaction (American Psychiatric Association, 2013). Epidemiological studies indicate that ADHD is a prevalent disorder affecting between 6.7 to 7.8% of children worldwide (Thomas, et al., 2015). This prevalence is higher in Arabic speaking countries reaching

9.4% in Egypt (Bishry, et al., 2014) and 11.6% in Saudi Arabia (Homidi, et al., 2013). Based on family history, genotyping, and neuroimaging studies, there is clear evidence to support a biological basis for ADHD. Although multiple regions of the brain and several neurotransmitters have been implicated in the emergence of symptoms, dopamine continues to be a focus of investigation regarding ADHD symptoms (Sadock, et al., 2014). Inadequate levels of norepinephrine and dopamine in brain synapses have been implicated as a cause of ADHD symptoms (Dervola, et al., 2012). The disturbance in dopaminergic function is considered as a possible cause of ADHD that alters serotonergic and glutamatergic transmissions (Tripp, et al., 2012).

Central nervous system stimulants are considered the first choice of treatment for ADHD as they have been shown to have the best efficacy. Non-stimulant medications approved by the United States, Food and Drug Administration (FDA) in the treatment of ADHD include atomoxetine, a norepinephrine uptake inhibitor. Unlike the stimulants, atomoxetine carries with it a black box warning for potential increases in suicidal thoughts or behaviors and requires children with ADHD to be monitored for these symptoms. α-agonists, clonidine and guanfacine, are also FDA approved for the treatment of ADHD, but for patients aged 6 years and older. Moreover, the UK National Institute for Health and Care Excellence(NICE) guidelines does not recommend prescribing any of these medications in children younger than 6 years old (Sadock, et al, 2014).

Cognitive abilities may be affected by deficient PUFA in the diet of children, and its supplementation to pregnant women may affect the offspring (Millichap and Yee, 2012), and potentially affect the prevalence of ADHD (Dopheide and Pliszka, 2009). Pre-and postnatal environmental factors play an important role in the pathogenesis of ADHD. Under nutrition and dietary deficiency in ADHD patients has been proposed to be one of postnatal factors. An imbalance ratio between omega-3 and omega-6 intake has been suggested to be involved in the development of ADHD (Raz and Gabis, 2009). Moreover, dietary supplementation with omega-3 PUFA may enhance both synaptic development and function (Kim, et al, 2011). Gene transcription, neurotransmitter metabolism and activities of synaptic vesicles and transporters may be changed by different fatty acids in neurons and glia cells (Levant, et al, 2006). Several regulatory processes such as modification of cerebral receptors in certain brain areas were changed as a result of omega-3 PUFA deficiency as this leads to changes in the vesicular pool and synaptic levels of serotonin (5-HT) (Chalon, 2006). Docosahexaenoic acid (DHA) –one of omega-3 fatty acids – promotes glutamatergic synaptic activities with subsequent increases in synapsin and glutamate receptor subunit expression in hippocampal neurons (Kim, et al., 2011). As a result, spontaneous synaptic activity is significantly intensified. Deficient of DHA results in inhibition of synaptogenesis, decreases in synapsins and glutamate receptor subunits, and long-term potentiation of hippocampal neurons impairment (Cao, et al., 2009).

Although role for brain neurotransmitters in the etiology of ADHD has been hypothesized for decades, yet measuring levels of neurotransmitters has received limited research attention, and to the best of our knowledge this is the first research studying the neurotransmitters' status in Egyptian children (less than 6 years old) diagnosed with ADHD. This is an aspect of the problem which if highlighted may provide a better understanding of the psycho pathophysiology of patients with ADHD. Moreover, effectiveness of PUFA as treatment of ADHD is controversy. Results of different researchers studied effectiveness of PUFA are contradicted from studies showed that PUFA is as effective as stimulants to studies found that it causes no improvement at all in ADHD symptoms. Hence, the current study also aimed to investigate the potential effects of PUFA supplementation on levels of these neurotransmitters and to study the efficacy of PUFA coupled with vitamins supplementation in alleviating ADHD symptoms in children younger than 6 years old.

## MATERIALS AND METHODS

### Patients recruitment

This pilot study recruited 40 children diagnosed with ADHD. They were selected from children with special needs clinic– National Research Centre NRC (Cairo, Egypt). A written informed consent was obtained from the guardians of the participants, according to the guidelines of the ethical committee of the National Research Centre, Cairo, Egypt (all experiments were approved by the Institution Ethical Review Board according to Helsinki Declaration 1964 revised in 2013). ADHD patient with co-morbid psychiatric disorders, on treatment for ADHD, with below

average IQ of 70 or with chronic medical illness were excluded.

**Procedures**

1) At the beginning of the study (baseline), all patients were subjected to the following assessments (see Box 1):

Full personal and medical history with thorough clinical examination. A three-generation family pedigree were constructed and analyzed to check for other affected family members.

Clinical psychiatric interviewing: ADHD was diagnosed according to DSM-5(American Psychiatric Association, 2013).

Mini International Neuropsychiatric Interview for Children (M.I.N.I. Kid) which is a short structured diagnostic interview for DSM-IV and ICD-10 psychiatric disorders (Sheehan and Janavs, 1998). This interview was employed to confirm ADHD diagnosis and to exclude other psychiatric co-morbidities. The Arabic version of M.I.N.I. Kid has been validated with satisfactory reliability and validity and has been used in many studies in Arab Countries (Ghanem, et al., 1999).

IQ assessment using the Arabic version of Stanford-Binet Intelligence Scale, 5th Edition (SB5) has been employed (Farag, 2011) to exclude children with below average IQ (70).

2) ADHD patient were further assessed using the Arabic version of Conners' Parent Rating Scale-Revised; Long Version (CPRS-R-L) to assess the severity of ADHD symptoms (Conners, 1997; El-Sheikh, et al, 2003).

3) At the end of study period the ADHD patients were assessed using CPRS-R-L to assess the response to treatment.

4) Laboratory investigations:

Plasma level of Serotonin (5-HT) using the High-Performance Liquid Chromatography (HPLC) method

Plasma level of Norepinephrine (NE) using HPLC method

Plasma level of Dopamine (DA) by HPLC method

Plasma level of γ amino butyric acid (GABA) (ELISA)

Plasma level of Glutamate (using colorimetric method)

Levels of these neurotransmitters were re-measured at the end of study period.

**Box 1: Summary of all pre- and post-intervention tests for ADHD patients**

| **Test** | **ADHD patient** | |
|---|---|---|
| | **Pre-intervention** | **Post-intervention** |
| **Full personal and medical history with thorough clinical examination** | Yes | - - |
| **DSM-5** | Yes | - - |
| **M.I.N.I. Kid)** | Yes | - - |
| **IQ assessment** | Yes | - - |
| **Conners' Parent Rating Scale-Revised** | Yes | - - |
| **Long Version (CPRS-R-L)** | Yes | Yes |
| **Laboratory tests** | | |
| **Serotonin (5-HT)** | Yes | Yes |
| **Norepinephrine (NE)** | Yes | Yes |
| **Dopamine (DA)** | Yes | Yes |
| **γ amino butyric acid (GABA)** | Yes | Yes |
| **Glutamate** | Yes | Yes |

**Box 2: Nutrition facts of major constituents of intervention meal (*values are given per 100g SSDP weight*)**

| **Macronutrients (g/100g)** | **Micronutrients (g/100g)** |
|---|---|
| **Total solids =** 29.2g<br>**Total protein =** 12 g | **Minerals** *(g/100g)*<br>Phosphorus 0.0196g<br>Potassium 0.0097g<br>Calcium 0.0260g<br>Magnesium 0.0097g<br>Manganese 0.000036g<br>Zinc 0.000357g |
| **Fat =** 15.5 g<br>**PUFA =**3.1g<br>**Omega-3 =** 2.65 g | |
| **Sugar** *(g/100g)*<br>Lactose (4.2g)<br>Sucrose (15g) | **Vitamins** *(g/100g)*<br>All-trans-retinol 0.000635g<br>Retinylpalmitate0.000421g<br>Vitamin C 0.00798g |
| **Calories** 263.1 kcal/100g | |
| Serving Size (50g) given once per day | |

**HPLC operation conditions:**

The determination of norepinephrine, dopamine and serotonin was carried out using laboratory HPLC system, Agilent technologies 1100 series, equipped with a quaternary pump (Quat pump, G131A model). Separation was achieved on ODS-reversed phase column (C18, 25 x 0.46 cm internal diameter 5 µm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1.5 ml/min. UV detection was performed at 270 nm, and the injection volume was 20 µl. The concentration of both catecholamines and serotonin were determined by external standard method using calculated peak areas. Serial dilutions of standards were injected, and their peak areas were derived. A linear standard curve has been constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was quantified from the curve.

**Nutritional intervention program**

Semi-solid dairy product (SSDP) was prepared from Buffalo's milk retentate which contained 29.2% total solids, 15.5 %, fat and 12 % total protein, in addition to 3.1% PUFA, essential minerals, vitamin A and vitamin C. Box-2 shows all actual values of minerals and vitamins of SSDP, including the total calorific value and total weight of the given semi-solid dairy product. Additionally, to their normal dietary intake each patient consumed 50g of SSDP on daily basis. SSDP has been supplemented to patients for the duration of six months during which the ADHD children were regularly followed up every two-week interval in the clinic to provide them with the supplement, monitor intake and assess if any adverse effect that might emerge. At the end of these six months there was post intervention assessment of neurotransmitters to verify the impact post-nutritional intervention program. This was coupled with re-assessment of these children using Conners' Parent Rating Scale-Revised; Long Version.

**Statistical tests:**

SPSS computer package version 17 (SPSS, Chicago, IL, USA) has been employed to analyze the gained data and formulate data interpretation.

## RESULTS

**Sociodemographic data:**

Table (1) showed the socio demographic data (age, sex, place of residence).

**Table (1): Socio-demographics of ADHD patients**

| Socio demographic data | | ADHD patients N=40 |
|---|---|---|
| **Gender** | male | 28 (70%) |
| | female | 12 (30%) |
| **Age** | mean± SD | 5.1± 1.2 |
| | | |
| **Residency** | urban | 35 (89.2%) |
| | rural | 5 (10.8%) |

**Family profile and family history**:

Regarding consanguinity 21.6% of parents of ADHD patients were first cousins.

**Levels of neurotransmitters in ADHD patients before and after supplementations:**

Comparison of plasma levels of neurotransmitters in ADHD patients before starting the supplements and at the end of study period reported statistical significant increase in levels of dopamine, norepinephrine and GABA associated with significant decrease in level of glutamate (table 2).

**Table (2): Neurotransmitters' levels in ADHD patients before and after supplementation for six-month period (N=40)**

| Neurotransmitters | Before treatment Mean±SD | After treatment Mean±SD | P value |
|---|---|---|---|
| **Dopamine** (pg/ml) | 146.7± 46.7 | 238.7 ± 43.6 | **0.00*** |
| **Serotonin** (ng/ml) | 156.8± 50.5 | 160.4 ±69.43 | 0.4 |
| **Norepinephrine** (pg/ml) | 87.9± 7.7 | 114.5 ± 45.9 | **0.00*** |
| **Glutamate** (µg/ml) | 110.26± 17.44 | 95±12.20 | **0.05*** |
| **GABA (**pmol /ml) | 133.1± 21.5 | 319.1± 18.2 | **0.00*** |

pg/ml: pictogram per milliliter. ng/ml: nanogram per milliliter

µg/ml: microgram per milliliter. pmol /ml:picomol per milliliter

* Statistically significant differences $p \leq 0.05$

**Severity of ADHD symptoms in patients post-supplementation (six-month intervention):**

Comparison of severity of ADHD symptoms pre-and post-supplementation revealed statistically significant improvement on the inattention, DSM-IV: inattentive, DSM-IV: total and Conners' ADHD, restless-impulsive and emotional liability indices as shown in figure (1) and table (3).

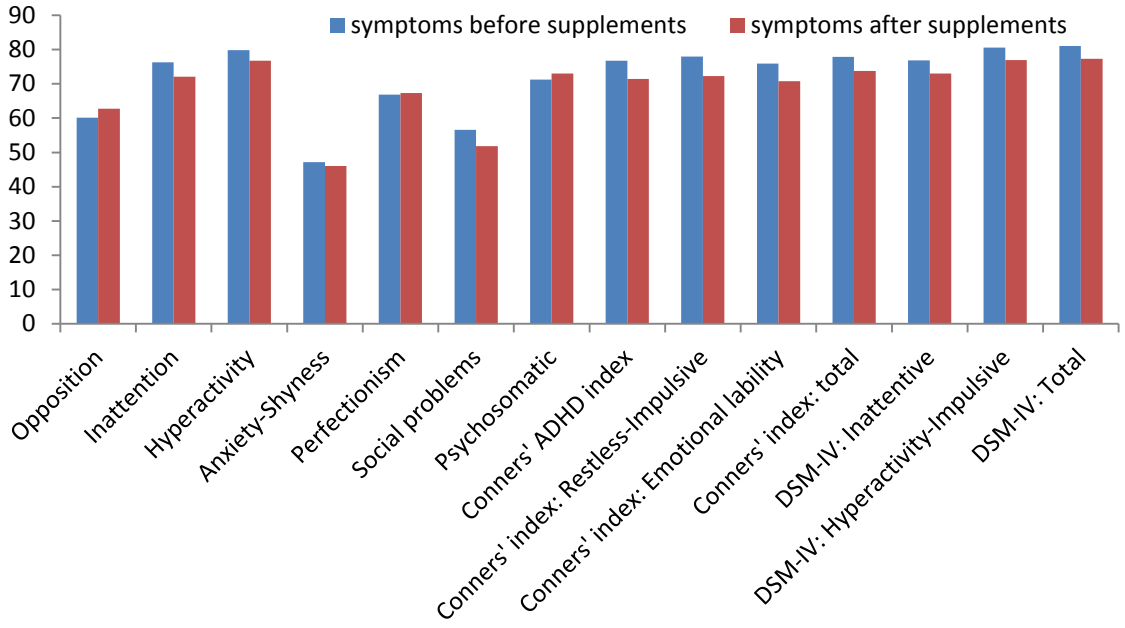


**Figure (1): Severity of ADHD symptoms, as assessed by Conners' Parent Rating Scale, before and after supplements**.

There was statistically significant improvement on the inattention (p value= 0.001), DSM-IV: inattentive (p value= 0.000), DSM-IV: total (p value= 0.003) and Conners' ADHD (p value= 0.000), restless-impulsive (p value= 0.02) and emotional lability (p value= 0.018) indices

**Table (3): Severity of ADHD symptoms as assessed by CPRS-R-L before and after supplements**

| Conner's parents rating scale | Patients before treatment | | Patients after treatment | | P value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| **Opposition** | 60.09 | 10.8 | 62.7 | 11.2 | 0.43 |
| **Inattention** | 76.3 | 11.201 | 72.03 | 14.44 | **0.001*** |
| **Hyperactivity** | 79.83 | 13.641 | 76.73 | 12.01 | 0.189 |
| **Anxiety-Shyness** | 47.2 | 2.5 | 46 | 1.6 | 0.25 |
| **Perfectionism** | 66.83 | 13.081 | 67.3 | 10.31 | 0.84 |
| **Social problems** | 56.55 | 10.40 | 51.82 | 9.93 | 0.076 |
| **Psychosomatic** | 71.2 | 18.874 | 73 | 13.85 | 0.565 |
| **Conners' ADHD index** | 76.73 | 9.172 | 71.43 | 11.78 | **0.000*** |
| **Conners'index:Restless-Impulsive** | 77.97 | 10.07 | 72.27 | 13.47 | **0.02*** |
| **Conners' index: Emotional lability** | 75.87 | 12.564 | 70.73 | 15.32 | **0.018*** |
| **Conners' index: total** | 77.87 | 11.1 | 73.77 | 14.31 | 0.07 |
| **DSM-IV: Inattentive** | 76.83 | 10.185 | 73.03 | 12.99 | **0.000*** |
| **DSM-IV:Hyperactivity-Impulsive** | 80.53 | 11.331 | 76.93 | 9.738 | 0.058 |
| **DSM-IV: Total** | 81.07 | 8.61 | 77.27 | 11.7 | **0.003*** |

* Statistically significant improvement on the domains of inattention, Conners' ADHD, restless-impulsive

and emotional lability indices, DSM-IV: inattentive and DSM-IV: total of the Conner's parents rating scale in the ADHD patients before and after the 6-months nutritional program as $p \leq 0.05$.

## DISCUSSION

In this study, role of genetic factor has been investigated, 21.6% of parents of ADHD patients were first cousins. Delivery by cesarean section was 40.5% of ADHD patients. This finding is consistent with the literature However, Tian et al., (2009) had concluded that cesarean section itself had no significant impact on attentiveness in school children, while medical indications for cesarean section may have contributed to a major cause of ADHD (Tian, et al., 2009).

### Supplementation with Omega-3 Fatty acids

The supplementation with PUFAs in childhood has a pivotal role for the functioning and development of the brain (Borsonelo and Galduróz, 2008), as the docosahexaenoic (DHA) and arachidonic acid (AA), accumulate rapidly in the gray matter of the brain during early childhood (Wainwright, 2002), their deficiency may lead to irreversible memory, learning, mood and sensorial system deficits (Yehuda, et al., 2005).

The present study indicated a possible improvement in the symptomatology of the ADHD, and showed statistical significant improvement in the following Conner's parents rating scale subscales; inattention, DSM-IV: inattentive, DSM-IV: total and Conners' ADHD, restless-impulsive and emotional lability indices after nutritional supplementation (1000 mg PUFA once daily for six months intervention), this is consistent with Germano et al.. (2007)

A diet deficient inomega-3 may influence neurotransmission, the dopaminergic and the serotonergic systems, which lead to reduction of dopamine (Chalon, 2006). The increase in the ingestion of omega-3 may promote the central activity of dopamine in the pre-frontal cortex, thus deceasing aggressiveness and impulsiveness (Chalon et al., 2001).

### Plasma level of neurotransmitters:

This study showed significant increase in norepinephrine and dopamine levels ($p=0.001$) and slight non-significant increase ($p=0.4$) in serotonin level pre- and post-nutritional intervention program.

The diet, chronic stress, nutritional deficiencies and environmental toxins were considered as primary risk factors for imbalance or depletion of neurotransmitters, but also genetic polymorphism may prevent the formation of certain neurotransmitters (Borsonelo and Galduróz, 2008).

The excitatory neurotransmitter glutamate implicated, in the pathophysiology of ADHD, due to its interaction with dopamine and norepinephrine (Lesch, et al, 2013) and imbalances in glutamate may interfere with the gating of sensory information in striato-frontal pathways in those patients.

Deficits in omega-3 fatty acids have been linked to many neurodevelopmental problems including ADHD, and its deficiency causes hyperactivity or lack of attention. ADHD children have reduced red blood cell omega-3 fatty acids compared to typically developing children. Deficits in dietary omega-3 fatty acid have also been linked to alterations in glutamatergic and serotoninergic neurotransmission, as well as mesocortical and mesolimbic dopaminergic system dysfunction (Borsonelo and Galduróz, 2008). Inflammatory mechanisms have been proposed to be possible factors in ADHD (Buske-Kirschbaum, et al., 2013). Nonetheless, PUFA has anti-inflammatory actions, so it can prevent inflammation in astrocytes (Singh, et al., 2012). It also influences genetic transcription, signal transduction (Haag, et al, 2003), and the fluidity of membranes, since the functional activity of the $Na^+/K^+$ ATPase is influenced by the fatty acid composition of the membrane these mechanisms may clarify glutamate uptake regulation (Gerbi, et al., 1999).

The reduction in GABA levels is accompanied with less cognitive control, impaired response inhibition and high impulsivity (Silveri, et al., 2013). In the present study the results showed significant increase in GABA (the main inhibitory neurotransmitter) level when compared to its level before intervention, on the other hand there was a significant decrease in glutamate level ($p$-value$<0.05$) as a result of the nutritional intervention program,.

## CONCLUSION

In conclusion, healthy and balanced nutrition should be encouraged among children who are 6 years old and living with ADHD. While pharmacological therapy has proven to be efficacious in controlling disruptive behavior and inattention in those patients; however, it is not recommended for children younger than 6 years old. In this study, nutritional supplementation with omega-3 fatty acids and selected vitamins appeared to be effective in alleviating ADHD

symptoms especially the cognitive problems and inattention in less than 6 years old children. Moreover, omega-3 and vitamins supplementation induces increase in plasma levels of dopamine, norepinephrine and GABA and as well as decrease in level of glutamate the excitatory neurotransmitter, so it should be considered more extensively in the management of ADHD patients, in particular this studied cohort.

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## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

NM designed the study, diagnosed and followed up the cases.MA and JH performed biochemical analysis and wrote the manuscript .AH performed sample collection and biochemical analysis. HH diagnosed, clinically assessed and followed up the cases. FS and MF developed the semi-solid meal. IT contributed to the writing, amending and approving of manuscript. AY reviewed the manuscript. All authors read and approved the final version.



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