Attention deficit hyperactivity disorder (ADHD) is a highly frequent childhood disease usually treated with neurotropic stimulant drugs, therapy with serious side effects especially for children with neurologic comorbidities. Considering the low levels of essential fatty acids in children’s serum with this disorder, the present study evaluated the effects of dietary omega-3 fatty acids supplementation in ADHD children with epilepsy. 17 children with epilepsy and ADHD have been clinically evaluated concerning neurological, psychiatric, psychological, EEG, biochemical (urinary catecholamines) parameters. They received supplements with omega-3 fatty acids for 6 months (with re-evaluation of the biochemical markers and the psychological tests-Conners questionnaire). 14 children accomplished the study. All patients had high scores of ADHD questionnaires before adding-on the dietary supplement and after 6 months, 75% showed a significant improvement, by measuring the symptoms of inattention and impulsivity, academic and language difficulties. Regarding the catecholamines’ (namely adrenaline, noradrenaline and dopamine), our results showed a significant decrease of their levels in urine, compared to baseline. Regarding the behavioural benefit in combination with the low risk due to a good safety profile, the dietary supplementation with omega-3 fatty acids offers a promising complementary approach to standard therapy.

Rezumat

Tulburarea de hiperactivitate cu deficit de atenție (ADHD) este o boală foarte frecventă a copilului, de obicei tratată cu medicamente stimulante neurotrope, terapie cu efecte secundare grave, în special pentru copiii cu comorbidități neurologice. Având în vedere nivelurile serice scăzute de acizi grași esențiali ale copiilor cu această afecțiune, studiul prezent a evaluat efectele suplimentării dietei cu acizi grași omega-3 la copii cu ADHD cu epilepsie. 17 copii cu epilepsie și ADHD au fost evaluăți clinic, în ceea ce privește parametrii neurologici, psihiatrici, psihologici, EEG, biochimici (catecolamine urinare). Pacienții au primit suplimente cu acizi grași omega-3 timp de 6 luni (cu reevaluarea markerilor biochimici și a testelor psihologice - chestionarul Conners). 14 copii au finalizat studiul. Toți pacienții au avut scări semnificative a nivelurilor lor urinar, comparativ cu valoarele inițiale. În ceea ce privește beneficiile la nivelul comportamentului pacienților cu ADHD, în combinație cu riscul scăzut datorat unui profil de siguranță bun, suplimentele alimentare cu acizi grași omega-3 oferă o abordare promițătoare complementară terapiei standard.

Keywords: attention deficit with hyperactivity disorder (ADHD), omega-3 fatty acids, epilepsy, catecholamines, urine samples

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a heterogeneous neuro-biological disorder determined by many genetic, environmental, psychosocial and familial factors [1], with high prevalence (3 - 7%) in young people worldwide [2]. It is characterized by a combination of hyperactivity, impulsive behaviour and lack of attention, pervasive features, all of these symptoms being persistent in time. The patients are diagnosed after multiple evaluations, including anamnestic data (parents and teachers), clinical
examinations (general, psychiatric, neurological), psychological evaluation (specific testing for ADHD performed by a psychologist) and paraclinical testing (blood tests, EEG and cerebral imagistic exam for differential diagnosis for associated diseases to ADHD). Regarding the association between ADHD and epilepsy, many questions are still under debate [3, 4]. From the theoretical perspectives, is ADHD associated with epilepsy the same as the ADHD seen in patients who do not have epilepsy? More specifically, do children with epilepsy with ADHD have primary attention deficits or secondary deficits associated with slowing of processing speed, subtle to severe cognitive impairments [5], and language-related learning disorders [6]? Epilepsy variables are not related to the inattentive type of ADHD (I-ADHD) found in youth with epilepsy who are of average intelligence [5]. The early age of onset, poor seizure control, and the number of antiepileptic drugs, however, are reported in subjects with epilepsy and intellectual disability who also have high rates of the combined and hyperactive/impulsive types (H/I-ADHD) of ADHD [7]. The association of these epilepsy variables with low intelligence quotient (IQ), therefore, underscores the need to determine if ADHD associated with epilepsy is secondary to the cognitive and language deficits described earlier. An exhaustive management plan should include appropriate educational behavioural measures and intervention, together with the standard pharmacological treatment. The standard pharmacological treatment may be neurostimulating or non-neurostimulating, the most frequently prescribed being metilphenidate, a drug similar to amphetamines as mechanism and duration of effect. It is assumed to act by activating the ascending reticular system to brainstem and the cerebral cortex. When it is given in therapeutic doses, it has a mild to moderate stimulating effect. In hyperactive children it has a paradoxical, relaxing effect. Clinical studies have shown that the long-term use of methylphenidate leads to moderate adverse reactions in most patients, but also some severe ones in others (decreased appetite, insomnia, headaches) and therefore it should be used with caution and only with specific recommendations [2, 4, 6-9]. The family of ADHD patients is usually reserved and suspicious about the administration of psychostimulant medication to the child, which is another reason why dietary supplements have begun to be studied in this field. Of these, the administration of essential fatty acids supplements (omega-3 in particular) has been shown to be effective. Another alternative treatment is the cognitive behavioural therapy, which is more effective if it is combined to drug treatments [3-5, 7, 10].

In recent years, there has been a great interest in the study of essential fatty acids, especially regarding the promising role of omega-3 in neurodevelopment, neurocognition and neurodegenerative disorders [11]. There are promising researches on the role of omega-3 in the prevention of neuropsychiatric disorders [12].

ADHD aetiology seems to be linked to the presence of proinflammatory markers [13] and low levels of poly unsaturated fatty acids (PUFAs) [14]. ω-3 fatty acid, docosahexaenoic acid (DHA), is involved in synaptic integrity and synaptic neurotransmission, the depletion of specific synaptic proteins due to brain DHA-depletion being an important mechanism for the suboptimal brain function associated with ω-3 fatty acids deficiency [15]. The greatest impact of essential fatty acids deficiency is during the development of the nervous system, because they contribute to the normal development of the nervous system. To clarify the hypothesis that supplements containing PUFAs can be effective in ADHD, it is important to understand their role for the healthy brain, namely the production of phospholipids in neuronal membranes, as well as the fluidity and permeability of the neuronal membrane [16]. High concentrations of omega-3 are found especially at the synaptic level, in order to have a flexible and a maximum potential of the neuronal functionality. Several studies have been conducted in children and adolescents and showed low concentrations of essential fatty acids in patients’ plasma diagnosed with ADHD compared with the control group, demonstrating the potential effect of omega-3 and omega-6 fatty acids in improving the symptoms of ADHD [17]. Also the PUFAs levels are correlated with alpha-2 adrenergic receptors expression [18] or the oligomerization kinetics of dopamine D2 receptors [19]. Dietary supplementation with omega-3 fatty acids led to the decrease of plasma inflammatory mediators and oxidative stress biomarkers [20]. Young people diagnosed with ADHD present high levels of catecholamines [21, 22] and urinary levels of adrenaline (ADR), noradrenaline (NA) or dopamine (DA) were correlated with the disease evolution [23]. The current study aims to demonstrate the clinical effectiveness of omega-3 fatty acids (DHA and eicosapentaenoic acid (EPA)) administration as a dietary supplement for ADHD in patients with epilepsy (multiple benefits of omega-3 compared to virtually absent side effects) together with their involvement in the catecholamines metabolic pathways.

Materials and Methods

The current study resulted from a collaboration between the Paediatric Neurology Clinic of the Alexandru Obregia’s Psychiatric Hospital and the Faculty of Pharmacy, Bucharest, Romania. The prospective study extended over a six months period (between 2015 and 2016) and enrolled children from our
clinic after their legal guardians signed the informed consent. The study was approved by the Institutional Ethics Committee.

Study design

Physicians at the Paediatric Neurology Clinic of the Alexandru Obregia’s Psychiatric Hospital (paediatric neurology and paediatric psychiatry) dealt with the selection of children diagnosed with epilepsy (concordant with the new definition of epilepsy from 2014) and ADHD (The Diagnostic and Statistical Manual of Mental Disorders (DSMV) criteria for the diagnosis of ADHD). The inclusion criteria were concordant with the diagnostic criteria. The task force of International League Against Epilepsy proposed that epilepsy should be considered as disease of the brain defined by any of the following conditions: (1) at least two unprovoked (or reflex) seizures occurring > 24 h apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; (3) diagnosis of an epileptic syndrome [24].

Physicians interviewed the parents for concluding the diagnosis of ADHD, evaluated the type of epilepsy, performed the general clinical examination, neurological and mental status, EEG exam and noted the type of antiepileptic drugs administered. The psychologist applied the specific tests for ADHD and monitored the progress during omega-3 supplementation, comparing the Conners 3 scores in the two evaluations 6 months apart (before and after 6 months of supplementation with omega-3 fatty acids). The duration of the study was 6 months, and the selection of patients enrolled in the study was in accordance with the inclusion and exclusion criteria.

The inclusion criteria in the study were: (i) age: 6 - 18 years; (ii) diagnosis of epilepsy; (iii) non-progressive evolution; (iv) six or more symptoms of the DSMV diagnostic criteria for ADHD that persisted for at least 6 months to a degree that was inconsistent with the developmental level, and negatively impacts directly on social and academic/occupational activities. It is to be noted that the symptoms are not solely a manifestation of oppositional behaviour, defiance, hostility or failure to understand tasks or instructions [25].

Exclusion criteria for patients in the study were: (i) age < 6 years; (ii) not epilepsy; (iii) progressive neurologic disease; (iv) mental disorders not concordant with ADHD; (v) neurological background treatment with psychiatric effects.

Initial assessment was performed by the paediatric neurologist, paediatric psychiatrist and psychologist and included: (i) interview with parents/family, for assessing the family history (epilepsy, febrile convulsions, ADHD, anxiety disorders, schizophrenia, personality disorders), child history (antecedents related to pregnancy and birth, psychomotor development by age, other neuropsychological or other associated pathologies), epilepsy history (onset, evolution, performance-related school information, verbal/written reports from educators/teachers, antiepileptic treatments, and the result of these treatments); (ii) clinical evaluation included basic information on height, weight, vital signs, neurological examination and mental status assessment, behaviour doctor office, family; (iii) the paraclinical assessment considered screening, urine analysis, standard EEG and cerebral imaging examinations.

Psychological assessment was performed by an authorized psychologist using the Conners 3 test. This test contains multiple scales that evaluate not only ADHD, but also related issues such as executive functioning, learning problems, aggressiveness, relationship with family and peers. These scales can be applied to young people between the ages of 8 and 18 (self-evaluation) and to parents and teachers who evaluate young people aged 6 to 18 (hetero-evaluation). For the initial evaluation, the long Conners 3 form was used (it offers the most detailed and complex information). The scores were calculated at baseline, and then after 6 months of treatment. The psychologist summed the scores from each area of the test and assigned the raw scores to the correct age group column within each scale. The scores were then converted to standardized scores, known as T-scores. T-scores were also converted into percentile scores. Percentile scores can help us to see how severe the child’s ADHD symptoms are compared to other children’s symptoms. Lastly, the T-scores were put into a graph form so that they can interpret them visually: T-scores above 60 are usually a sign that the child may have an emotional, behavioural, or academic problem, such as ADHD; T-scores from 61 to 70 are usually a sign that the child’s emotional, behavioural, or academic problems are slightly atypical, or moderately severe; T-scores above 70 are usually a sign that the emotional, behavioural, or academic problems are very atypical, or more severe.

As with all psychological evaluation tools, the Conners CBRS has its limitations. Those who use the scale as a diagnostic tool for ADHD run the risk of incorrectly diagnosing the disorder or failing to diagnose the disorder. Experts recommend using the Conners CBRS with other diagnostic measures, such as ADHD symptom checklists and attention-span tests.

Sample collection and catecholamines assay

Patients collected 24 h urine samples in containers with 15 mL of 6 M HCl, as a preservative. The volumes were measured and 5 mL of the urine samples were stored at -20°C until analysed. Adrenaline (ADR), noradrenaline (NA) and dopamine
(DA) were assayed using a competitive enzyme immune assay with a commercial ELISA kit (MyBio Source) using microtiter plates. The analytical sensitivity of the assay specified by the manufacturer was 0.9 ng/mL for ADR, 1.7 ng/mL for NA and 2.5 ng/mL for DA. The cross reactivity of several substances with similar chemical structure was < 0.1 percent.

**Dietary supplementation.** It was used sea fish oil 1070 mg (250 mg DHA and 54 mg EPA/daily), which was a kind gift from Quisser Pharma (Doppelherz® system Omega-3 Junior Syrup) as add-on to the antiepileptic drugs scheme (which was stable during the study).

**Follow-ups** were performed at 3 months and 6 months after the initiation of treatment by the neurologists, psychologists and pharmacists. After 3 months, the follow-up included clinical assessment (general and neurological somatic examination), interview with parents - reports, ideas, observations made by parents and exposed to repeated visits, as well as possible adverse reactions (digestive - nausea, transit disorders, increased risk of bleeding when combined with anticoagulants) and urinary catecholamines evaluation. After 6 months, the follow-up included the same evaluation as for 3 months plus the psychological re-assessment performed by the same authorized psychologist with the Conners 3 questionnaire. The assessment tools were once more applied by the psychologist in order to be able to object to the effects of the administered supplement.

**Statistical analysis**

Results are expressed as mean ± standard deviation. Statistical analysis was performed with GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The applied statistical tests (Student’s t test, ANOVA, Pearson correlations) have a 90% confidence interval (CI90%). Statistical significance was considered for p < 0.05.

**Results and Discussion**

**Clinical evaluation**

17 children and adolescents diagnosed with epilepsy and ADHD were enrolled in the initial assessment, considering the clinical exam performed by the neurologist and psychiatrist, and also the interview with parents. 3 of them were invalidated by the Conners 3 test (because of incomplete assessment done by the patient/parent due to the lack of understanding the questions), so only 14 patients completed the study (6 - 14 years old, 13 boys and one girl). 92.8% of children had focal epilepsy associated with ADHD: 60% of them had structural epilepsy (epileptic seizures related to cerebral structural abnormality), resistant to treatment, and 40% had genetic focal epilepsy (almost all with partial seizures control, only one child is seizure free without antiepileptic drugs). 85.7% of children with focal epilepsy and seizures resistant to treatment or partially controlled had at least 1 - 2 seizures/month. One child (7.1%) had generalized epileptic encephalopathy resistant to treatment.

71% of the subjects had associated neurological or psychological disorders: 6 children with associated cerebral palsy (unilateral pyramidal form secondary to premature birth or hypoxic-ischemic encephalopathy at term birth) and 4 children had language disability. For epilepsy, 90% of children received at least 2 antiepileptic drugs, different combinations between: valproic acid, carbamazepine, clobazam, topiramate, lamotrigine, nitrazepam, ethosucinimide, sulthiam (therapeutic blood levels). During the dietary supplementation with ADHD there was no need to change/modify the antiepileptic scheme for any of the participants.

EEGs showed active epileptiform discharges in almost 80% of children (in 30% as epileptic encephalopathy with continuous spike-wave complexes during slow-wave sleep).

There were no side effects to omega-3 supplementation for any of the studied patients; the syrup was very well tolerated/accepted, considering its pleasant taste. All 14 patients accomplished the study; there was no case of early study terminating. First results were at 3 months after initiating the dietary supplementation with omega-3 fatty acids. The data were obtained from the neurological and psychological clinical examination and the interview with parents/patients. Almost all patients (85.7%) showed significant improvement regarding the lack of attention, hyperactivity, opposition/defiant behaviour. Parents noticed symptoms relieve and also at the medical control the children were quieter/more obedient, cooperative, less distracted by stimuli, with increased attention. On the question of school activity (learning, memorizing), parents report from teachers showed a slight improvement. The patients ADHD clinical evaluations before and 6 months after 250 mg DHA and 54 mg EPA administration are depicted in Figure 1.

The results pointed out that 6 months after the dietary supplementation with omega-3 fatty acids, the severity of the symptoms presented a trend of amelioration. The decrease was significant (p < 0.05) for the inattentiveness and hyperactivity/impulsivity. At the initial psychological evaluation, the median score for ADHD was T-score > 70, with significant improvement after 6 months of treatment with omega 3 (almost 65% have T-score < 50) and 21% obtained T-score between 50 and 60). Only one patient had a slight increase of T-score from 72 to 80, and 1 patient had almost the same score as the initial phase.
Figure 1.
ADHD patients’ clinical status (a: Conner’s CBRS scales; b: DSM-IV-TR Symptom scales)

Table I
Urinary levels of ADR, NA and DA

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Initial ADR (nmol/L)</th>
<th>Initial NA (nmol/L)</th>
<th>Initial DA (nmol/L)</th>
<th>3 months ADR (nmol/L)</th>
<th>3 months NA (nmol/L)</th>
<th>3 months DA (nmol/L)</th>
<th>6 months ADR (nmol/L)</th>
<th>6 months NA (nmol/L)</th>
<th>6 months DA (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>3 months</td>
<td>6 months</td>
<td>Initial</td>
<td>3 months</td>
<td>6 months</td>
<td>Initial</td>
<td>3 months</td>
<td>6 months</td>
</tr>
<tr>
<td>ADR</td>
<td>2.83 ± 0.26</td>
<td>2.56 ± 0.25</td>
<td>2.13 ± 0.20</td>
<td>7.15 ± 0.59</td>
<td>6.74 ± 0.56</td>
<td>6.61 ± 0.55</td>
<td>46.56 ± 2.11</td>
<td>42.44 ± 1.83</td>
<td>32.52 ± 2.00</td>
</tr>
<tr>
<td>NA</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td>NR</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>DA</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II
The correlation between the ADHD symptoms severity and catecholamine concentrations (Pearson correlation coefficient, r)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Initial</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADR</td>
<td>DA</td>
</tr>
<tr>
<td>Inattentive</td>
<td>0.31</td>
<td>-0.06</td>
</tr>
<tr>
<td>Hyperactivity/Impulsivity</td>
<td>0.46</td>
<td>0.32</td>
</tr>
<tr>
<td>Academic difficulties</td>
<td>0.32</td>
<td>-0.24</td>
</tr>
<tr>
<td>Conduct disorder</td>
<td>0.55</td>
<td>-0.08</td>
</tr>
<tr>
<td>Violence potential</td>
<td>0.33</td>
<td>-0.22</td>
</tr>
<tr>
<td>Social problems</td>
<td>-0.15</td>
<td>0.24</td>
</tr>
<tr>
<td>ADHD inattentive</td>
<td>0.36</td>
<td>0.04</td>
</tr>
<tr>
<td>ADHD hyperactive/impulsive</td>
<td>-0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Conduct disorder</td>
<td>0.06</td>
<td>0.60</td>
</tr>
<tr>
<td>Oppositional defiant disorder</td>
<td>-0.11</td>
<td>0.44</td>
</tr>
</tbody>
</table>

* Statistical significance

Biochemical analysis
The results of the urinary levels of ADR, NA and DA are presented in Table I. The patients’ treatment supplemented with DHA and EPA led to a decrease of the assessed catecholamines concentrations at the end of the observation period, with statistical significances depicted in Table I. The correlation between the ADHD symptoms severity and catecholamines’ concentrations is presented in Table II.

The Pearson statistics indicates no correlations between ADHD symptoms and catecholamine’s concentrations before the supplementation with omega-3 fatty acids. It was observed a statistical significant correlation between the amelioration of the ADHD patients conduct disorder symptoms and the decrease of the DA levels at the end (6 months) of the observation period (r = 0.90). Weak correlations were observed between Hyperactivity/Impulsivity and Academic difficulties symptoms and the decrease of ADR levels.

The research team will review the patients after 12 months (6 months without omega-3) to see if the response to omega-3 supplementation is consistent in time or not.

ADHD is a chronic disease where stimulant pharmacotherapy is an efficient evidence-based treatment of choice, but being a chronic disease, it is important to consider other complementary and alternative
therapies, with fewer side-effects [26]. Considering the behavioural benefit in combination with the low risk due to a good safety profile, the dietary supplementation with omega-3 acids can be recommended [27] in these patients.

Given their relative safety and general health benefits, supplementation with omega-3 fatty acids offers a promising complementary approach to standard therapy, so more studies are needed in the future [28].

Study limitations
Considering the data presented, the number of patients which was not very large and the high number of variables considered (different aetiologies, types of epilepsy, EEGs aspects and antiepileptic drugs), it is difficult to draw a conclusion as cause-effect, but the omega-3 fatty acids seemed to be efficient for ADHD associated to seizure disorder in children. This study can be a start-up for large controlled placebo trials to confirm these good results of omega-3 and to try to find specific factors for the clinical response.

Conclusions
The dietary supplementation of the treated patients enrolled in the study, with PUFAs led to the improvement of the ADHD clinical status. The decrease of the catecholamines’ urinary levels appears to be linked to the fatty acids dietary supplementation. Moreover, the decreases of dopamine levels were correlated with the improvement of paediatric patients conduct disorder.

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Conflict of interest
The authors declare no conflict of interest.

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