By L-CARNITINE LEVEL IN CHILDREN WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER

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ABSTRACT

Background: *Throughout the past era, numerous studies reported the association between attention deficit hyperactivity disorder (ADHD) and deficiency of magnesium, zinc, selenium, and vitamins A, D, E, B complex, and essential free amino acids. However, the role of carnitine deficiency in the development on ADHD has not been investigated extensively.*

Methods: *This is a cross-sectional study which involved 2 groups, a case group of 30 patients and a matching control group of 30 subjects. We employed a spectrophotometric enzymatic assay to quantify total-carnitine using Human L-Carnitine ELISA Kit (96 T Unit).*

Results: *An overall 60 patients who fulfillment the inclusion criteria were included in the study. Patients were furtherly assorted into ADHD (case) group (30 patients) and healthy control group (30 participants). The mean age of the included patients was 7.1296±1.76 and 7.0417±2.05 among the case and control groups, respectively (p=0.19). As for serum carnitine level, there was no statistically significant difference between control and patients as regards serum carnitine (p=0.792). Comparison between serum carnitine and the different clinical subtypes of ADHD indicated non-significant difference between them as regards serum carnitine level (Predominantly Hyperactive: 75.9+19.0; Predominantly inattentive: 62.5+35.96, and Combined ADHD 54.5+25.4, F-ratio=0.827, p>0.05).*

Conclusions: *It does not seem that carnitine deficiency is an essential cause for development of ADHD symptoms, but its supplementation in ADHD children has clinically proven beneficial effect that may be attributed to one or more of its various functions in the brain.*

Keywords: *Attention deficit hyperactivity disorder, Carnitine, Deficiency.*

INTRODUCTION

Introduction Attention deficit hyperactivity disorder (ADHD) is a childhood-onset neurodevelopmental disease which characterized by a wide range behavior, social, mental and a considerable intellectual disability [1]. The estimated prevalence of ADHD is nearly 4% among children with under reporting of the actual incidence in developing countries [2].

In Egypt, the reported incidence of ADHD in primary schools ranged from 6.5 to 8% [3]. ADHD has multiple repercussions across many aspects of patient's life comprising academic, social, occupational functioning and quality of life [4]. Besides that, it can affect the lives of parents and family members by putting multiple strains on their public relationships [5].

ADHD is highly heritable, and many factors participated in the development of it. In details, ADHD may develop as a sequel of multiple genes and non-inherited factors coupled with prenatal and perinatal factors; on the contrary, definite causes remain unknown [6].

Throughout the past era, numerous studies reported the association between ADHD and deficiency of magnesium, zinc, selenium, and vitamins A, D, E, B complex, and essential free amino acids [7, 8, 9]. However, the role of carnitine deficiency in the development on ADHD has not been investigated extensively [10]. In this concern, brain carnitine deficiency might cause autism as an eventual effect of TMLHE deficiency - a defect in carnitine biosynthesis [11]. In particular, a gene on the X chromosome (SLC6A14) likely escapes random X-inactivation (a mixed epigenetic and genetic regulation) and could limit carnitine transport across the blood-brain barrier [12]. The current study was established to assess the role of L-Carnitine deficiency in ADHD development among sample of Egyptian children who admitted at Al-Hussein University and Sayed Galal University Hospitals.

PATIENTS AND METHODS

Ethical Approval:

This study was implemented based upon the approved ethics of the ethical research board (ERB) of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt along with the recommendations of the Declaration of Helsinki. Prior to study proceeding, all patients assigned informed consents after the obvious explanation of the possible

adverse events. The informed agreements were gained from the legal trustee of the included children.

Study Design:

This study is a cross-sectional study, which has been executed at the pediatric and psychiatry Departments, Al-Hussien and Sayed Galal University Hospitals, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

The research sample involved 2 groups, a case group of 30 patients and a matching control group of 30 subjects.

I. Case Group:

An overall 30 children with ADHD were selected from the outpatients clinic of pediatric and psychiatry departments, by convenient sampling of new cases coming for consultation, whose parents consented to participate their children in the study, in a period from March 2018 to June 2019.

A. Inclusion Criteria:

1. Male or female, age range 5-12 years.
2. The child meets DSM-IV criteria for ADHD.
3. Newly diagnosed case and has not received treatment yet.
4. An informed consent from the child's parents.

B. Exclusion Criteria:

1. Children suffering from any chronic medical condition, as diabetes, that was diagnosed before the start of study.
2. Patients with other neurological disorder or on antiepileptic drugs.
3. Combined Psychiatric disorder.

II. Control Group:

A total of 30 matched healthy volunteer children with no history suggestive of neurodevelopmental disorder or any chronic medical illness. The children in this group are matched for age and sex.

Method:

I. Clinical assessment:

Children of the case and control group were subjected to the following test(s):

1. Full history taking by consultant child psychiatrist.
2. Clinical examination by consultant child psychiatrist.
3. Conner's' Parent Rating Scale (CPRS).
4. Informed consent from parent(s).
5. Blood sample collection.

II. Laboratory Method:

Single blood sample of 2 millilmeters were withdrawn aseptically into a sterile disposable syringe from each subject. The sample was allowed to clot for 30 minute before spinning in a centrifuge for a 15 minute at approximately 1000 x g and was stored at -20°C. We employed a spectrophotometric enzymatic assay to quantify total-carnitine using Human L-Carnitine ELISA Kit (96 T Unit).

Apparatus used: Beckman DU 3436 Hu Spectophotometer Made in England.

Statistical Methods:

Statistical analysis was performed using SPSS software version 23 for Windows (SPSS Inc., Chicago, IL, USA), and MedCalc software version 14.8 (MedCalc Software, Mariakerke, Belgium).

Continuous normally distributed parameters were explicated in the form of mean, and standard deviation (SD), and its specific groups were compared using student t-test. Conversely, non-normally distributed data were expressed using median and range and were compared using Man Whitney U test. Categorical variables were illustrated using the number and percentage and its particular groups were compared using Pearson's chi-square test with Fisher's exact test.

RESULTS

Table (1): Demographic characteristics of studied groups

	Case n.=30	Control n.=30	P.V
Age (years)	**7.13 ± 1.6**	**7.04 ± 2.05**	**0.19**
Sex			
Male	**18 (60%)**	**22 (73.3%)**	
Female	**12 (40%)**	**8 (26.7%)**	

This table shows no significant difference regarding age between the two studied groups.

Table (2): Sub types of ADHD using DSM IVin studied groups

Subtype	Number	(%)
Hyperactive	**4**	**13.3%**
Inattentive	**8**	**26.6%**
Combined	**18**	**60%**

This table shows that the combined type of ADHD was the commonest as in the study.

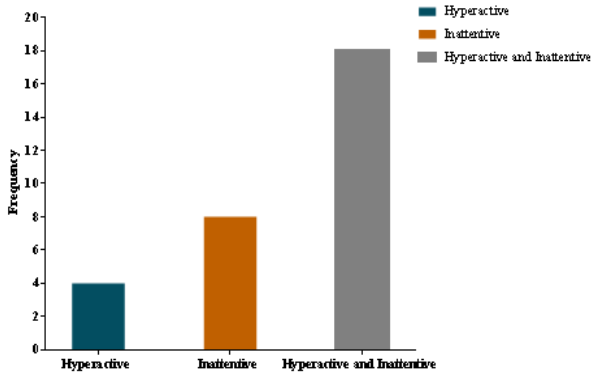

Figure (1): Bar chart delineated the pattern of ADHD subtypes among the case group

Laboratory Findings:

Table (3): The levels of L. carnitine among the studied groups

	L carnitin level mg/dl						
	Min.	**Max.**	**Mean**	**SD**	**p**	**Z**	**Sig.**
Control (n. 30)	41 mg/dl	72.5 mg/dl	60.8542	±9.041	0.792	-0.264	NS
Pts (n. 30)	17 mg/dl	110 mg /dl	58.9204	±27.78			

Figure.2 and Table.3 show no significant difference between studied groups regarding Lcarnitine level.

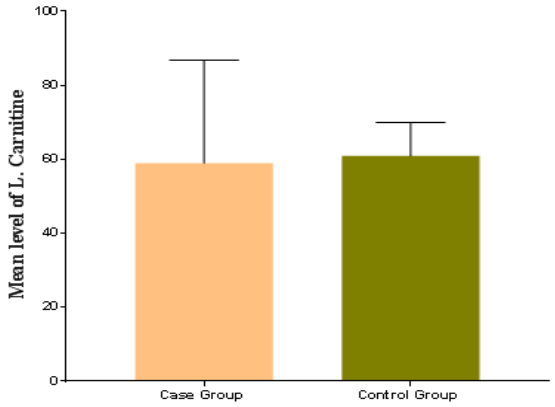

Figure (2): Error bar chart showed the differences of mean level of L. carnitine among the studied groups

Correlation analysis between L. Carnitine levels and ADHD.

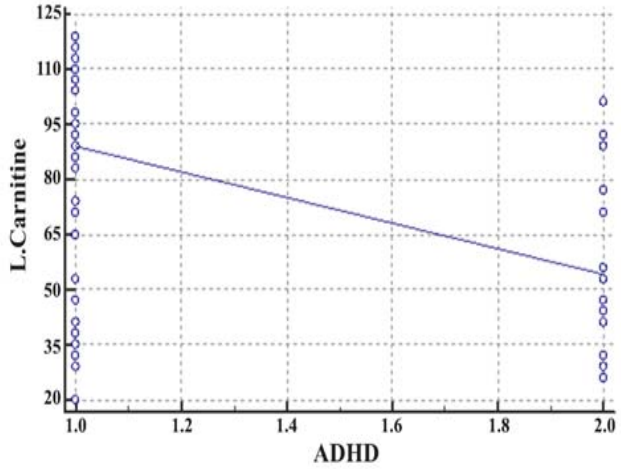

Figure (3): Scatter plot showed the correlation between the L. carnitine levels and presence of ADHD

Table (4): Correlation between serum carnitine and the different subtypes of ADHD

	Hyperactive		Inattention		Combined ADHD		F		P
	Mean	SD±	Mean	SD	Mean	SD±			
Carnitine mg/dl	75.9333	± 19.01482	62.4643	± 35.96191	54.4588	± 25.37033	0.827	2	0.449

This table shows no significant correlation between serum carnitine and the different subtypes of ADHD.

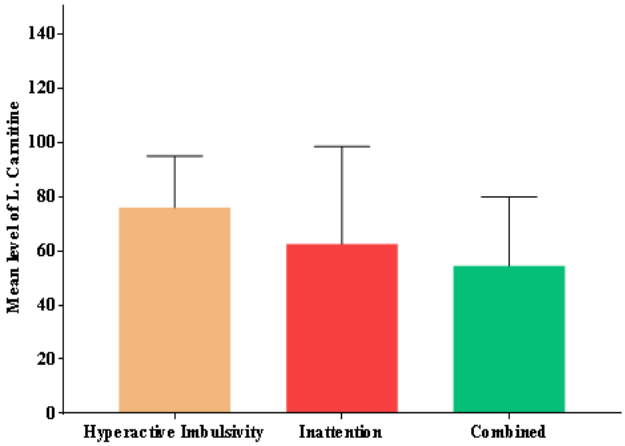

Figure (4): Error bar chart showed the mean levels of carnitine among ADHD subtypes

DISCUSSION

Attention-deficit/hyperactivity disorder (ADHD) is one of the most commonly diagnosed behavioral disorders of childhood. The disorder is estimated to affect between 3 and 7 out of every 100 school-aged children]. This makes ADHD a major health concern. The disorder does not affect only children. In many cases, problems continue through adolescence and adulthood [13].

Plenty of literature has been discussing functions of carnitine in the brain and its possible role in various psychiatric and neurological disorders [10, 14, 15]. However, the diagnostic role of L. Carnitine in ADHD did not be handled adequately. Thereafter, this study aimed to compare serum carnitine level among a sample of children with ADHD and children who do not have this disorder.

The attention-deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder of with a spectrum of problematic behavior, possibly caused by structural or functional abnormalities in the connections between the frontal cortex and the basal ganglia. It is the most common psychiatric disorder in children and the symptoms often persist into adolescence and adulthood [16, 17].

Some investigators consider ADHD and its comorbidity, as a disorder of fatty acid and phospholipid metabolism. It is of interest that the concentration of this fatty acid, which is important for brain maturation and functioning, is decreased in

plasma of ADHD patients [18, 19]. More interestingly, decreased levels of serum carnitine (P>0.001) were observed in a large sample (n = 100) of autistic patients by [20].

By combining all these scientific evidence obtained in the present study, the hypothesis of our study comes directly forward that serum L-carnitine level did not relate to the presence, phenomenology or the severity of ADHD. However, more knowledge about serum carnitine level would help to understand more about this hypothesis.

There is substantial evidence that maternal genotype and carnitine levels determine the carnitine status of infants at birth, with asymptomatic mothers with biallelic systemic primary carnitine deficiency leading to transient severe, but apparently benign, carnitine deficiency in their heterozygous unaffected offspring [21, 22]. Newborns with biallelic systemic carnitine deficiency are occasionally detected by newborn screening, but the reliability is unknown, and positive tests more often result from maternal than from infant deficiency [10].

The importance of nutrition during the first 1,000 days (conception through age 2 years) is recognized as especially important for growth and development [10]. However, there is no evidence of in utero deficiency, and the content and bioavailability of carnitine in breast milk, infant formulas, and cow's milk likely provide protection in the first 3–6 months of life. Carnitine deficiency might simply reduce mitochondrial copy number generally [23]. Although the focus here is on carnitine deficiency as a primary form of pathogenesis, the same pathophysiology could act as a modifier for Mendelian or other forms of autism.

On contrary to the results of our study, Mostafa et al. noted dramatically low levels of carnitine and polyunsaturated fatty acids (PUFA) and elevated lactate levels in autistic children in Cairo between 4 and 12 years of age. After ten years of follow-up, this group again reported strikingly low levels of carnitine and PUFA, including docosahexaenoic acid (DHA), this time in children from Saudi Arabia [24]. The degree of laboratory abnormalities and the older age of the children suggest the possibility that an extremely deficient diet in an older age group might reveal biochemical findings that might be only transient on a different diet. The discrepancy between the results of our study

and Mostafa et al study might be attributed to the difference in the study design and sample characteristics, whereby our study is a cross-sectional study and Mostafa et al study is a prospective study.

Despite the evidence summarized in the current investigation, some limitations may hinder its evidence. Of note, the cross-sectional design, limited sample size, and the lack of adequate follow-up periods which limited our capability to evaluate the long-term sequences of L-Carnitine deficiency.

CONCLUSION

It does not seem that carnitine deficiency is an essential cause for development of ADHD symptoms, but its supplementation in ADHD children has clinically proven beneficial effect that may be attributed to one or more of its various functions in the brain. Thereafter, children with ADHD seem to be in more need for carnitine functions, than for carnitine itself. Further studies are with a randomized design and considerable number of patients to tackle the limitations of the present study.

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مستوى ل – كارنيتين للاطفال المصابين باضطراب فرط الحركه و قصور الانتباه

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خلفيـــة الدراســـة: خـــلال العقـــد الماضـــى، أفـــادت العديـــد مـــن الدراســات عــن وجــود علاقــة بــين اضــطراب فــرط النشــاط ونقــص الانتبـــاه ونقـــص المغنيســـيوم والزنـــك والســـيلينيوم والفيتامينـــات والأحمــاض الأمينيــة الأساســية. ومــع ذلــك، لــم يــتم التحقيــق علــى نطــاق واســع دور نقــص الكــارنيتين فــي حــدوث اضــطراب فــرط النشاط ونقص الانتباه.

المرضـــى و الطـــرق: هـــذه الدراســـة المستعرضـــة شـــملت مجمـــوعتين، مجموعـــة مـــن مرضـــى اضـــطراب فـــرط النشـــاط ونقــص الانتبــاه (30مريضــا) ومجموعــة ســليمة مطابقــة مــن 30 شخصــا. اســتخدمنا مقايســة إنزيميــة طيفيــة لقيــاس الكــارنيتين الكلــي باستخدام مجموعة أدوات ELISA.

النتـــائج: تـــم تضـــمين مـــا مجموعـــه 60 مريضـــا الـــذين يســـتوفون معــايير الاشــتمال فــي الدراســة. تــم تنويــع المرضــى بشــكل إضــافي فـــي مجموعـــة مرضـــى اضـــطراب فـــرط النشـــاط ونقـــص الانتبـــاه (30 مريضًـــا) ومجموعـــة ســـليمة مطابقـــة صـــحياً (30 مشـــاركًا). كـــان متوســـط عمـــر المرضـــى المشـــمولين 7.1296 ± 1.76 و 7.0417 ± 2.05 بـــين مجموعـــات المرضـــى والســـليمة، علـــى

التوالي (ع = 0.19). بالنسبة لمستوى الكارنيتين في الدم، لم يكن هناك فرق ذي دلالة إحصائية بين مرضى اضطراب فرط النشاط ونقص الانتباه والمجموعة السليمة فيما يتعلق بنسبة الكارنيتين (ع = 0.792). أشارت المقارنة بين الكارنيتين في الأنواع الفرعية السريرية المختلفة من اضطراب فرط الحركة ونقص الانتباه إلى عدم وجود إختلاف إحصائى بينهما فيما يتعلق بمستوى الكارنيتين (الغالب مفرط النشاط: 75.9 + 19.0؛ غالبًا غير مدرك: 62.5 + 35.96، و ADHD 54.5 + 25.4 +، ونسبة ADHD 54.5 + 25.4، F- نسبة = 0.827، ص> 0.05).

الاستنتاجات: لا يبدو أن نقص الكارنيتين هو سبب أساسي لتطور أعراض اضطراب فرط الحركة ونقص الانتباه، ولكن مكملاته في أطفال اضطراب فرط الحركة ونقص الانتباه قد أثبتت سريريًا تأثير مفيد قد يعزى إلى واحد أو أكثر من وظائفه المختلفة في الدماغ.