Serum Level of L-carnitine in a Sample of Children with Attention-Deficit Hyperactivity Disorder

Thesis

for partial fullfilment of Master Degree

in Neuropsychiatry

By

Mohamed Hamdy Abdelkader Ataalla

M.B. Ch.B.

Supervised by

Prof. Dr. Afaf Hamed Khalil

Proffesor of Psychiatry

Faculty of Medicine, Ain Shams University

Ass. Prof. Hanan Hussein Ahmad

Assist. Proffesor of Psychiatry

Faculty of Medicine, Ain Shams University

Ass. Prof. Hanan M. Ezz Eddin Azzam

Assist. Proffesor of Psychiatry

Faculty of Medicine, Ain Shams University

Ain shams University

Faculty of Medicine

Cairo 2010

INTRODUCTION

Attention-Deficit Hyperactivity Disorder (ADHD) is a neurobehavioral developmental disorder ***(Zwi et al., 2000))*** affecting about 3-5% of the world's population. It typically presents during childhood, and is characterized by a persistent pattern of impulsiveness and inattention, with or without a component of hyperactivity***(American Psychiatric Association, 2000***). ADHD occurs twice as commonly in boys as in girls ***(Dulcan, 1997)***. ADHD is generally a chronic disorder with 10 to 60% of individuals diagnosed in childhood continuing to meet diagnostic criteria in adulthood ***(Cleave& Leslie, 2008).*** Many researchers are still struggeling for a better understanding of the pathogenesis of ADHD,that will certainly help to offer a safer and more effective lines of treatment of a prevelant child psychiatric disorder.

Attention deficit/hyperactivity disorder (ADHD) actually seems to have heterogeneous origins ***(Johansen et al. 2002; Solanto 2002).*** These considerations derive from the notion that heterogeneous neurobiological alterations may lead to similar observed symptoms. Several pathogenetic hypotheses have been proposed, including: dysfunctions in the mesolimbic system or its inhibitory regulation by the pre-frontal cortex (PFC) ***(Johansen et al., 2002)***; altered overproduction and pruning of dopamine (DA) receptors in the PFC ***(Andersen and Teicher, 2000);*** and altered serotonergic and/or noradrenergic metabolism ***(Oades, 2002).*** Specifically, some symptoms of impulsivity could be ascribed to an altered serotonergic function (***Linnoila et al., 1983***; ***Soubrie' 1986).*** Peripheral levels of the 5-HIAA serotonin metabolite are increased ***(Castellanos et al., 1994)*** and the dopamine-metabolite/serotonin-metabolite ratio is lower in ADHD versus control children, suggesting a hyperactive serotonergic system ***(Oades, 2002)***.

In one study, a delay in development of certain brain structures by an average of three years occurred in ADHD elementary school aged patients. The delay was most prominent in the frontal cortex and temporal lobe, which are believed to be responsible for the ability to control and focus thinking. In contrast, the motor cortex in the ADHD patients was seen to mature faster than normal, suggesting that both slower development of behavioral control and advanced motor development might be required for the fidgetiness that characterize an ADHD diagnosis. Brain matures a few years late in ADHD, but follows normal pattern ***(Rapoport, 2007).***

However,although there are evidences for dopamine and growth abnormalities in ADHD, it is not clear whether these abnormalities are of the molecular abnormality of ADHD or a secondary consequence of a problem elsewhere. It is assumed that oxidative stress could play a role in this ***(Dvoráková et al., 2006).*** In some neurological disorders,accumulation of toxic amyloid-beta (Abeta)-peptide is suggested to cause oxidative stress in the brain, and decrease the content of polyunsaturated fatty acids (PUFA) in neuronal membrane lipids ***(Petursdottir et al., 2007)***.

Several studies have identified abnormalities in membrane fatty acids in some subjects with ADHD, and some success has been reported using lipid therapies ***(Ross et al., 2003).*** Animal studies have shown that a deficiency in brain of the n-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) is associated with memory loss and diminished cognitive function ***(Petursdottir et al., 2008).*** Moreover,the gender difference, or male predominance, in ADHD was suggested to be due to protection against oxidative stress by the female steroid hormone,estrogens, acting as antioxidants ***(Sawada and Shimohama, 2000)***. L- Carnitine and its ester, acetyl-l-carnitine (ALC), facilitate the transport of long chain free fatty acids across the mitochondrial membrane enhancing neuronal anti-oxidative defense ***(Alves et al., 2008)***.

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids (or fats) for the generation of metabolic energy. It is often sold as a nutritional supplement. Carnitine was originally found as a growth factor for mealworms and labeled vitamin B. Carnitine exists in two stereoisomers: its biologically active form is L-carnitine, while its enantiomer, D-carnitine, is biologically inactive ***(Liedtke et al., 1982***).

Carnitine transports long-chain acyl groups from fatty acids into the mitochondrial matrix, so that they can be broken down through β-oxidation to acetate to obtain usable energy via the citric acid cycle. In some organisms such as fungi, the acetate is used in the glyoxylate cycle for gluconeogenesis and formation of carbohydrates. Fatty acids must be activated before binding to the carnitine molecule to form acyl-carnitine. The free fatty acid in the cytosol is attached with a thioester bond to coenzyme A (CoA). This reaction is catalyzed by the enzyme fatty acyl-CoA synthetase and driven to completion by inorganic pyrophosphatase ***(Olpin,2005)***. ALC is an energy reservoir ***(Aureli et al., 1998),*** which is

converted through deacetylation to carnitine. The latter is a carrier of long-chain fatty acids, which are important for brain maturation and functioning ***(Salvati et al., 2000)***. ALC may increase the reservoir of activated acetyl groups, which are involved in the reacylation of membrane phospholipids ***(Virmani et al. 1995).*** Different carnitine concentrations and composition patterns in the brain may be related to maturation of the potential carnitine reserve and to metabolic functions, such as fatty acid utilization and the reservoir of acetyl units, in each developmental tissue ***(Nakano et al., 1989).*** In turn, long-chian polyunsaturated fatty acids have proved essential for brain growth and development ***(do Nascimento & Oyama,2003).***

In addition to that, carnitines exert a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress ***(Cavazza,2002)*** ALC may prevent cellular energy deficits and limit the formation and escape of superoxide radicals from mitochondria ***(Hagen et al., 2002; Virmani et al. 2002; Beal 2003).***

Different researches have investigated and tried to prove role of carnitine, or its deficiency, in the etiology of some neurological disorders, and whether it could be used to halt the progression of symptoms. Clinical trials utilizing ALC have shown beneficial effects on nerve conduction slowing, neuropathic pain, axonal degenerative changes and nerve fiber regeneration, despite relatively late initiation in the natural history of diabetic peripheral neuropathy. Owing to the good safety profile of ALC, early initiation of ALC therapy would be justified, with potentially greater benefits ***(Sima,2007)***. L-carnitine enhances resistance to oxidative stress by reducing DNA damage in Ataxia telangiectasia cells ***(Berni et al., 2008)***. Another research has investigated the role of carnitine and fatty acid oxidation and its defects in infantile epilepsy ***(Tein, 2002).***

Recently, researchers started to investigate how we can apply what we already know about carinitine in the management of ADHD. ADHD comorbidity includes disorders of fatty acid metabolism (***Richardson & Ros, 2000),*** their plasma concentration being decreased in ADHD patients ***(Stevens et al., 1995***). ALC administration has been shown to modulate positively aggressive behavior and attentional problems of ADHD children ***(Van Oudheusden and Scholte 2002),*** to reduce hyperactivity in fragile-X boys (***Torrioli et al., 1999),*** and to improve cognitive performance in rats (***Caprioli et al. 1990, 1995; Ghirardi et al. 1992).***

Rational for the study:

With an increasing awareness of parents of the mental health of their children, more and more cases of children with ADHD are presented to hospitals and child psychiatry clinics. Even at schools, the disruptive behavior of children with ADHD, has been a growing problem that led to increasing numbers of students being referred for psychiatric consultation. The DSM IV-TR suggests that the prevalence rate of ADHD in children is 3% to 7%. Using these prevalence rates it can be estimated that in a classroom of 25 to 30 children, at least one of those children will have ADHD.

Today, it is not questionable how severe or how much prevalent is the problem, but the question is how to provide an effective and safe lines of treatment of ADHD.

It is well established that methylphenidate (MPH) decreases impulsivity and increases sustained attention in humans ***(Ward et al., 1997)***. However, psychostimulant drug use is not without side effects and problems ***(Klein-Schwartz 2002; Rapport and Moffitt 2002)***. Great effort is devoted to the identification of novel non-psychostimulant agent. Acetyl- L-carnitine (ALC) has been proposed as a possible alternative. However, the use of carnitine in the treatment and its role in the pathogenesis of ADHD is still debatable and needs further direct assessment.

Hypothesis:

Serum L-carnitine level may have a role in pathogenenic or a pathoplastic effect on ADHD symptoms.

Aim of Work:

1- To compare serum L-carnitine level between ADHD and control children.

2-To correlate serum level of L-carnitine to the severity and type of ADHD symptoms.

SUBJECTS & METHODS:

SUBJECTS:

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

[I] Case Group:

30 children will be selected the Institute of Psychiatry, Ain Shams University, and at the Abbasia Hospital of by checking new cases diagnosed as ADHD in the Child Psychiatry outpatient Clinic at Mental Health. All newly diagnosed cases whose parents consent to participate in the study will be involved.

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.
5- Those who have IQ above 90.

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.

[II] Control Group:

30 matched healthy volunteer children with no history suggestive of neurodevelopmental disease or any chronic medical illness. The children in this group are matched for age, sex, IQ and social standards.

[III] Selection of Cases:

Cases will be recruited from the Child Psychiatry outpatient Clinic of the Institute of Psychaitry,Ain Shams University and the Child Unite at Abbasia Hospital of Mental Health .

METHOD:

- **Study design:**
Cross-sectional case control study.

[I] Clinical Method:
All children of the case group will be subjected to the following tests :
- **MINI-kid** Arbic version ***(*Ghanem.,*2000*)*** will be used to confirm the children who meet diagnostic criteria for of ADHD, and to detect any existing comorbidity .
- **Conner's' Parent Rating Scale (CPRS)** Arabic version (***El Sheikh, 2003***) will be used to assess the severity of the disease.
-**IQ** estimation, measured by psychologists, using similarities and block design subtest of Wechsler Intelligence Scale for Children, WISC Arabic version ***(Melka and Ismail,1980).***

These tests wil be applied as well to the control group.

[II]Lab Method:
- Setting:
Single blood sample will be withdrawn from every child in the case and control group. The sample will be clotted and the child should be fasting at the time of the sampling. Samples will then be sent to the Medical Research Center of Ain Shams University, where the readings of L-carnitine serum level will come out using the ELISA technique.

[III] Tools:
1-Syringe for blood sampling.
2-Kits for the determination of L-carnitine in blood samples.

[IV] Data management and analysis plan:
Results of the study will be statistically analyzed by the computerized version of statistical package for social sciences (SPSS).

<u>Ethical Considerations:</u>

1-Ethical considerations:

No pressure will be practiced on any parent to involve a child in the study. And all the data will be treated with confidentiality.

2-Informed consent:

The whole procedure of serum L-carnitine sampling will be explained to the parents, and will be assured that it will not expose the children to any

hazards. Also,a written consent will be obtained from the parents, and they will be provided by any positive results that are relevant to their children`s cases.

References

Aureli T, Di Cocco ME, Puccetti C, et al: (1998) Acetyl-L-carnitine modulates glucose metabolism and stimulates glycogen synthesis in rat brain. Brain Res 796:75–81.

Alves E, Binienda Z, Carvalho F, et al: (2008) " Acetyl-l-carnitine provides effective in vivo neuroprotection over 3,4-methylenedioximethamphetamine-induced mitochondrial neurotoxicity in the adolescent rat brain. " Neuroscience. [Epub ahead of print]

American Psychiatric Association : ***(2000)*** Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV). Washington DC: American Psychiatric Association,.

Andersen SL, Teicher MH : ***(2000)*** Sex differences in dopamine receptors and their relevance to ADHD. Neurosci Biobehav, Rev 24:137–141.

Beal MF: (2003) Bioenergetic approaches for neuroprotection in Parkinson's disease. Ann Neurol 53:S39–S47

Berni A, Meschini R, Filippi S, , et al: (2008) " L-carnitine enhances resistance to oxidative stress by reducing DNA damage in Ataxia telangiectasia cells. " Mutat Res. Feb 29;650(2):165-74.

Caprioli A, Ghirardi O, Ramacci MT, et al: (1990) Agedependent deficits in radial maze performance in the rat: effect of chronic treatment with acetyl-L-carnitine. Prog Neuropsychopharmacol Biol Psychiatry 14:359–369

Caprioli A, Markowska AL, Olton DS: ***(1995)*** Acetyl-L-carnitine: chronic treatment improves spatial acquisition in a new environment in aged rats. J Gerontol Biol Sci Med Sci 50: B232–B236

Castellanos FX, Elia J, Kruesi MJ, et al: (1994) Cerebrospinal fluid monoamine metabolites in boys with attention deficit hyperactivity disorder. Psychiatry Res 52:305–316.

Cavazza Claudio :(2002) "Composition for the Prevention and Treatment of Osteoporosis due to Menopause Syndrome", US Patent 6,335,038, column 3.

Cleave J, Leslie LK. :(2008) "Approaching ADHD as a chronic condition: implications for long-term adherence". Journal of psychosocial nursing and mental health services 46 (8): 28–37. PMID 18777966.

do Nascimento CM, Oyama LM : (2003) "Long-chain polyunsaturated fatty acids essential for brain growth and development " Nutrition. 2003 Jan;19(1):66 . PMID: 12507642 [PubMed - indexed for MEDLINE].

Dulcan M: (1997) "Practice parameters for the assessment and treatment of children, adolescents, and adults with attention-deficit/hyperactivity disorder. American Academy of Child and Adolescent Psychiatry". J Am Acad Child Adolesc Psychiatry 36 (10 Suppl): 85S–121S. PMID 9334567.

Dvoráková M, Sivonová M, Trebatická J, et al , ***: (2006)*** "The effect of polyphenolic extract from pine bark, Pycnogenol on the level of glutathione in children suffering from attention deficit hyperactivity disorder (ADHD)". PMID: 16984739 [PubMed - indexed for MEDLINE], Redox Rep. 2006;11(4):163-72

El Sheikh M., Sadek A.,Omar Abd. ,et al :(2003) "Psychiatric morbidity in first degree relatives of a sample of ADHD children" In an unpuplished MD thesis, Ain Shams University)

Ghanem M., Ibraheem M., ,et al :(2000) "**MINI-kid** Arbic version" (In an unpuplished MD thesis, Ain Shams University)

Ghirardi O, Giuliani A, Caprioli A, et al: (1992) Spatial memory in aged rats: population heterogeneity and effect of levocarnitine acetyl. J Neurosci Res 31:375–379**.**

Hagen TM, Liu J, Lykkesfeldt J, et al: (2002) Feeding acetyl-Lcarnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. Proc Natl Acad Sci USA 99:1870–1875.

Johansen EB, Aase H, Meyer A, et al: (2002) Attention deficit hyperactivity disorder (ADHD) behaviour explained by dysfunctioning reinforcement and extinction processes. Behav Brain Res 130:37–45.

Klein-Schwartz W: ***(2002)*** Abuse and toxicity of methylphenidate. Curr Opin Pediatr 14:219–223.

Liedtke A. J., Nellis S. H., Whitesell L. F. , et al: (1982). "Metabolic and mechanical effects using L- and D-carnitine in working swine hearts". Heart and Circulatory Physiology 243 (5): H691–H697. PMID 7137362.

Linnoila M, Virkkunen M, Schenin M, et al: (1983) Low cerebrospinal fluid 5- hydroxyindolacetic acid concentrations differentiate impulsive from non-impulsive violent behaviour. Life Sci 33:2609–2614.

Melka and Ismail : (1980) ."WISC Arabic version" Al Nahdah Egyptian Library.

Nakano, C : Takashima, S : Takeshita, et al: (1989) "Carnitine concentration during the development of human tissues" Early-Hum-Dev. 1989 Apr; 19(1): 21-7

Oades RD (2002) Dopamine may be "hyper" with respect to noradrenaline metabolism, but "hypo" with respect to serotonin metabolism, in children with attention-deficit hyperactivity disorder. Behav Brain Res 130:97–102.

Olpin S (2005). "Fatty acid oxidation defects as a cause of neuromyopathic disease in infants and adults". Clin. Lab. 51 (5-6): 289–306. PMID 15991803.

Petursdottir AL, Farr SA, Morley JE, et al : (2008) " Effect of dietary n-3 polyunsaturated fatty acids on brain lipid fatty acid composition, learning ability, and memory of senescence-accelerated mouse". J Gerontol A Biol Sci Med Sci. 2008 Nov;63(11):1153-60.

Petursdottir AL, Farr SA, Morley JE, et al: (2007) "Lipid peroxidation in brain during aging in the senescence-accelerated mouse (SAM) " Neurobiol Aging. (8):1170-8. Epub 2006 Jul 17.

Rapoport Judith, Shaw : (2007) NIMH Child Psychiatry Branch, November 12.

Rapport MD, Moffitt C :***(2002)*** Attention deficit/hyperactivity disorder and methylphenidate. A review of height/weight, cardiovascular, and somatic complaint side effects. Clin Psychol Rev 22:1107–1131

Richardson AJ, Ross MA: (2000) Fatty acid metabolism in neurodevelopmental disorder: a new perspective on associations between attention deficit/hyperactivity disorder, dyslexia, dyspraxia and the autistic spectrum. Prostaglandins Leukot Essent Fatty Acids 63:1–9.

Ross BM, McKenzie I, Glen I, et al : (2003) " Increased levels of ethane, a non-invasive marker of n-3 fatty acid oxidation, in breath of children with attention deficit hyperactivity disorder" .Nutr Neurosci. Oct;6(5):277-81.

Sawada H, Shimohama S.:(2008) " Neuroprotective effects of estradiol in mesencephalic dopaminergic neurons " Neurosci Biobehav Rev. Jan;24(1):143-7.

Salvati S, Attorri L, Avellino C, et al: (2000): Diet, lipids and brain development. Dev Neurosci 22:481–487.

Sima AA .: (2007) "Acetyl-L-carnitine in diabetic polyneuropathy: experimental and clinical data" CNS Drugs;21 Suppl 1:13-23; discussion 45-6.

Soubrie' P (1986): Reconciling the role of central serotonin neurons in human and animal behaviour. Behav Brain Sci 9:319–364.

Stevens LJ, Zentall SS, Deck JL, et al: (1995): Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 62:761–768.

Torrioli MG, Vernacotola S, Mariotti P, et al: (1999): Double-blind, placebo controlled study of L-acetyl-carnitine for the treatment of hyperactive behaviour in fragile X syndrome. Am J Med Genet 87:366–368.

Tein I*. *(2002) : " Role of carnitine and fatty acid oxidation and its defects in infantile epilepsy" J Child Neurol. 2002 Dec;17 Suppl 3:3S57-82; discussion 3S82-3

Virmani MA, Biselli R, Spadoni A, et al: (1995): Protective actions of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors. Pharmacol Res 32:383–389.

Virmani A, Gaetani F, Imam S, et al: (2002): The protective role of L-carnitine against neurotoxicity evoked by drug of abuse, methamphetamine, could be related to mitochondrial dysfunction. Ann N Y Acad Sci 965:225–232.

Van Oudheusden LJ, Scholte HR (2002:) Efficacy of carnitine in the treatment of children with attention-deficit hyperactivity disorder. Prostaglandins Leukot Essent Fatty Acids 67:33–38.

Ward AS, Kelly TH, Foltin RW, Fischman MW (1997): Effects of Damphetamine on task performance and social behaviour of humans in a residential laboratory. Exp Clin Psychopharmacol 5:130–136.

Zwi M, Ramchandani P, Joughin C (2000): "Evidence and belief in ADHD". BMJ (Clinical research ed.) 321 (7267): 975–6. PMID 11039942. PMC: 1118810.

Acknowledgement

First and foremost I would like to express my thanks and deep appreciation to ***Prof. Afaf Hamed Khalil,*** Professor of Neuropsychiatry, Faculty of Medicine, Ain Shams University for her generous and continuous support, wise helpful guidance and motherly attitude. No words could describe my appreciation for her encouragement and support, and I will never be able to thank her sufficiently. It is great honor and pride to work under her guidance and supervision.

I am also eternally grateful and thankful to ***Dr. Hanan Hussein Ahmad,*** Assistant Professor of Neuropsychiatry, Faculty of Medicine, Ain Shams University for her helpful contributions, keen support, step by step supervision, and valuable instructions that made this work come out to truth.

I wish to express my sincere thanks and heartful gratitude to to ***Dr. Hanan Ezz Eddin Azzam*** Lecturer In Psychiatry, Faculty of Medicine, Ain Shams University for the great support, encouragement, patience and help to complete this work.

Also, I would like to express my deep thanks and appreciation to ***Dr. Heba El Shahawy,*** Assistant Professors of Neuropsychiatry, Faculty of Medicine, Ain Shams University, for her encouragement and advice during the early steps of this work.

Also, I would like to thank all those who did the lab work ,without them,this work could not be done, and to the Professors of the Biochemistry Department, Faculty of Medicine, Ain Shams University.

Last but not least, I wish to express my ultimate thanks and great gratitude to ***my family*** just for every thing.