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**Assessment Of Polyunsaturated Fatty Acids
(PUFAs), Carnitine, Lactate And Demographic
Factors In Autistic Children**

Thesis

Submitted for Fulfillment of Ph. D. degree in Childhood studies.
Medical Department(child health and nutrition)

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2005

Hamza MM, El-Shorbagy OE, El-Wakkad AS, El-Gamal HA and Mostafa GA. Assessment Of Polyunsaturated Fatty Acids (PUFAs), Carnitine, Lactate And Demographic Factors In Autistic Children. Thesis Submitted for Fulfillment of Ph.D. degree in Childhood studies . Medical Department (child health and nutrition) . Institute Of Postgraduate Childhood Studies. Ain-Shams University 2005.

ABSTRACT

Background:

Autism is the most severe psychiatric disorder of childhood. Indirect estimation of autistic children in Egypt expected to be from 6000 child up to 125.500 child and even more. Some researches theorize that autism as a phospholipid spectrum disorders . Polyunsaturated fatty acids (PUFAs) which include linoleic (LA) , alpha linolenic acids (ALA) (both of which must be supplied in the diet because they are not formed in the body) and others as arachidonic acid (AA) and docosahexaenoic (DHA) are needed for normal and healthy brain development and function especially during early infancy. They modulate membrane fluidity and hence function of the neuronal cells and they influence the function of powerful neurotransmitters. Human brain is 60% fat of which 25% is DHA which coats the receptor sites of the brain cells to modulate the retinal performance and specific brain functions such as memory, attending ability, speech and specific motor skills. A lack of DHA leads to developmental disorders. Also, Several direct or indirect observations have raised the possibility that some forms of autism may be due to impairment of mitochondrial function like that seen in mitochondrial encephalopathies

The aim of this study : was to measure plasma polyunsaturated fatty acids (DHA , Linolenic, Arachidonic and Linoleic acids) and mitochondrial function (through measuring plasma lactate and serum carnitine) in autistic children. Also some risk and sociodemographic factors in autistic children were studied.

Subjects and methods : So, this study was carried out on 30 autistic children in the outpatient special needs clinic in Institute of Postgraduate Childhood Studies, Ain-Shams University and outpatient pediatric clinic, Ain-Shams University in the period from the beginning of January 2004 to the end of December 2004. Their age ranged from 4 to 12 years old in comparison to 30 healthy children serving as controls. According to disease severity assessed by CARS, autistic children were classified into two groups.

Group I : (Patients with mild and moderate autism) It included 18 patients. *Group II :* (Patients with severe autism) It included 12 patients.

Patients were subjected to:

-Clinical history taking and neuropsychiatric evaluation. In addition, vaccination history (MMR) and developmental history were assessed. Assessment of socioeconomic status and risk factors for autism as maternal age at time of birth, season of birth and perinatal complications. And full neurological examination.

-Laboratory investigations: Serum carnitine , Plasma lactate and Plasma polyunsaturated fatty acids (PUFAs) which include Docosahexaenoic, Linolenic, Arachidonic and Linoleic acids and omega 6/omega 3 ratio was done till March 2005 at the National Research Center.

Results:

- There was significant difference between autistic patients and controls regarding the season of birth and the socioeconomic status being highest in spring season and above average social class.
- 33.3% of the autistic patients had family history of psychiatric disorders, on the other hand 10% of the controls had such disorders. The difference between both groups was significant ($P < 0.05$) .
- Autistic patients had significantly higher incidence of perinatal complications than controls ($P < 0.05$) .
- Autistic patients had significantly higher incidence of delayed developmental milestones including walking unassisted ($P < 0.01$), and very highly significant ($P < 0.0001$) delayed in mother recognition, stool and urine control than control group .
- Autistic patients had significantly ($P < 0.01$) higher incidence of sleep disorders, convulsions , gastrointestinal disturbances and hypotonia than controls.
- Severely autistic patients had significantly ($P < 0.05$) higher percent of sleep disorders than patients with mild and moderate autism.
- All autistic patients had significantly lower values of serum carnitine ($P < 0.0001$) and plasma PUFAs ($P < 0.01$) (except linoleic acid $P > 0.05$) and higher values of plasma lactate ($P < 0.001$) and $\omega 6/ \omega 3$ (AA/DHA) ($P < 0.05$) than controls.
- Patients with severe autism had significantly lower value of serum carnitine and higher value of plasma lactate than patients with mild and moderate autism ($P < 0.05$).
- The difference between male and female autistic patients regarding the studied laboratory markers was insignificant ($P > 0.05$) .
- There was highly significant negative correlation between DHA and $\omega 6/\omega 3$ among all autistic patients r^{**} is significant at $p < 0.01$.
- There was highly significant negative correlation between serum carnitine and plasma lactate among all autistic patients r^{**} is significant at $p < 0.01$.

- There was a significant negative association $p < 0.01$ between carnitine and lactate among autistic children. As twenty three out of the 27 patients with low serum carnitine levels (85.2%) had elevated plasma lactate as well. In addition, all the 3 patients (100%) with normal serum carnitine had also normal plasma lactate levels.
- Twelve autistic patients (40%) had history of regression i.e. they had regression in developmental milestones after a period of normal development. Patients with regressive autism had significantly higher percent of convulsions $p < 0.05$ and higher $\omega 6/\omega 3$ $p < 0.01$ than patients with non- regressive autism.
- Autistic patients with hypotonia had significantly lower plasma DHA $p < 0.01$ and higher $\omega 6/\omega 3$ $p < 0.05$ than autistic patients with normal tone.

In conclusion :

Brain energy metabolism of many autistic children is low due to summation of several factors including low plasma PUFAs, in addition to disturbed mitochondrial function as evidenced by decreased serum carnitine and increased plasma lactate levels.

Further studies are needed to prove these arguments and to investigate the etiology behind these disturbances, in addition to assess the effect of new therapeutic modalities for autism, as PUFAs, carnitine, and measures that augment the mitochondrial function on amelioration of the autistic phenotype.

INTRODUCTION

Autism is the most severe psychiatric disorder of childhood. Three main criteria are essential for its diagnosis which include; disturbed reciprocal social interaction, lack of communication and restriction of normal variation in behavior and interests (**Wolanezyk et al., 2001**). Raising a mentally deficient child is difficult, whereas raising an autistic child is heart breaking (**Rapin, 2000**).

Maturation of the brain and development of optimal cognitive capacity depend mainly on three essential factors which are genetic potential of the individual, environmental stimulation and availability of nutrients (**Galler et al., 1997**). Some cases of autism has been associated with several different organic conditions including brain energy metabolism disturbance which needs polyunsaturated fatty acids (PUFAs), carnitine and normal mitochondrial function (**Oliveira et al., 2005**) .

Polyunsaturated fatty acids (PUFAs) include linoleic (LA), alpha linolenic acids (ALA) (both of which must be supplied in the diet because they are not formed in the body) and others as arachidonic acid (AA) and docosahexaenoic (DHA) which are synthesized in the body through a combination of elongation and desaturation reactions (**Giovannini et al ., 1995**). They modulate membrane fluidity and hence function of the neuronal cell (**Vancassel et al.,2001**). PUFAs are needed for normal and healthy brain development and function especially during early infancy, and

they influence the function of powerful neurotransmitters (*Innis,2000*).

Human brain is 60% fat of which 25% is DHA which coats the receptor sites of the brain cells to modulate the retinal performance and specific brain functions such as memory, attending ability, speech and specific motor skills. A lack of DHA leads to developmental disorders (*Steven et al., 1996*). Some researches theorize that autism, attention deficit hyperactive disorder (ADHD), dyslexia and dysparaxia as a phospholipid spectrum disorders (*Richardson and Ross,2000*). Recently, it was reported that PUFAs supplementation constitutes an environmental factor able to alter the brain function (*Chalon et al., 2001*) as evidenced by the decrease in the antisocial behavior, including violence, with increase of PUFAs intake (*Gesh et al., 2002*).

Carnitine, a non essential nutrient synthesized in the liver and kidney, is essential for transport of long chain fatty acids across inner mitochondrial membrane for β - oxidation and energy production (*Virmani et al.,2002*). Synaptic transmission of multiple neurotransmitters needs the neurobiological effect of acetyl carnitine (*Traina et al., 2004*).

Several direct or indirect observations have raised the possibility that some forms of autism may be due to impairment of mitochondrial function like that seen in mitochondrial encephalopathies (*Lombard, 1998*). Mitochondrial defect, which could be assessed by measuring plasma lactate, may be the origin of the carnitine deficiency in

these autistic children (*Filipek et al.,2004*). So, strategies to augment the mitochondrial function by stimulating mitochondrial enzymes activity or decreasing the production of endogenous toxic metabolites may be beneficial in treatment of autism (*Lombard, 1998*).

Aim and Hypotheses of the Study

Hypotheses

Brain energy metabolism which needs PUFAs, carnitine and normal mitochondrial function is reported to be disturbed in some autistic patients. This may add a new therapeutic strategy in autism.

Aim of the Study

The aim of this work was to measure plasma essential fatty acids and mitochondrial function (through measuring plasma lactate and serum carnitine) in autistic patients. This may add new biological indices for autism and new therapeutic implications in terms of child nutrition (as supplementation of PUFAs and carnitine) in addition to strategies which augment the mitochondrial function. Also, some risk and sociodemographic factors in autistic children were studied.

AUTISM

Autism is the most severe psychiatric disorder of childhood (*Whitaker-Azmilia, 2001*). Autism spectrum disorder (ASD) is a group of diseases that is characterised by a delay in language development, impairment of social interaction and the use of restricted stereotyped patterns (*Jepson, 2002*). It is a neurally based psychiatric disorder but there is no consensus regarding the underlying neurofunctional abnormality (*Muller et al., 2003*). Autism develops before 36 mo of age and is typically diagnosable at 18 mo of age (*Dalton et al., 2004*).

HISTORICAL REVIEW

Leo Kanner pointed in 1943, the existence of a syndrome called early infantile autism. In 1956, *Kanner and Eisenberg* laid down two criteria for the definition of the disorder :

- 1- Profound lack of affective contact at least up to age 5 .
- 2-Elaborate repititive routines.

At that time there was a general belief that Kanner's classic autism was a unique and discrete condition. The concept of autism was found to be useful in clinical practice in which many practitioners who had lived or worked with autistic children were sure they could instantly identify any other child with the same type of handicap (*Waterhouse et al., 1989*).

In the decades that followed, however, it was difficult to reach consensus about the definition and validity of autism. Specifying and operationalizing the essential characteristics

necessary for a diagnosis took a much longer time than originally anticipated (*Sophie et al., 2002*).

Successive editions of the World Health Organization's "International Classification of Diseases" (ICD) and the American Psychiatric Association's "Diagnostic and Statistical Manual of the Mental Disorders" (DSM) have reflected changing ideas of autism and related disorders. Almost 50 years after Kanner's first description, the latest versions of ICD "Diagnostic and Statistical Manual of the Mental Disorders" (DSM). And DSM-IV (*American Psychiatric Association 1994*) provided virtually identical definitions of autism and autistic-like disorders (*Volkmar et al., 1994*).

In both systems the overall construct of autistic and autistic-like behavior problems has been given the name pervasive developmental disorders (PDD). The term "pervasive" was meant to emphasize that in autism development was disturbed over a range of different domains, in contrast to the relatively more delineated difficulties of the specific developmental disorders as the centrality of cognitive problems in mental retardation. The term "developmental" implies that individuals with these conditions suffer from disturbances in the normative unfolding of multiple developmental competencies, including social relations and communication. These disorders have their onset in the first years of life, and developmental disorders have important implications throughout the life span (*Sophie et al., 2002*).

PREVALENCE OF AUTISM

Autism was initially felt to be a fairly rare illness (less than 5 in 10,000). The first major epidemiologic survey of infantile autism was conducted by **Lotter 1966-1967** in England, in middlesex country. Lotter`s study found prevalence rate of children diagnosed as autistic 4.5 per 10,000. Another study was done by **Rutter 1978** in Scotland, showed prevalence rate almost the same 4.4/10,000.

But over the last twenty years, there has been an explosive increases in incidence, growing on average around 25% per year and up to 100% per year in some areas (**Jepson, 2002**).

Two projects one from France 1997, and the other one from Norway1998, used strict ICD10 Research Diagnostic Criteria. Both the French and the Norway groups reported a prevalence rate for autism of 1 per 2,000 person (**Petter and Tanguay, 2000**).

In the United states, it is currently believed to affect 1 out of every 250 individuals on average (up to 1 in 150 in some areas) with a 4 to 1 male predominance (**Jepson, 2002**). The prevalence of autism in a U.S. Metropolitan Area was 3-4 per 1000 (male to female ratio 4:1) and 68% of children with I.Q. or developmental test had cognitive impairment (**Allsopp et al., 2003**).

Recent studies show prevalence rates ranging from 10 to 20 per 10,000 children (*Dalton et al., 2004*).

In our country, no epidemiological studies have been done, but indirect estimation of autistic children in Egypt expected to be from 6000 child up to 125.500 child and even more (*Omar et al., 2001*). A study was done by the Department of Human Genetics National Research Center, about fragile-x (+ve) cases referred to the outpatient clinic which were 3.5% (6 out of 170 males with mental retardation) from year 85-88 (*Temtamy et al., 1988*).

CAUSES OF AUTISM

Rapin research 2000 has thoroughly discredited the theory that autism is an emotional disorder attributable to inadequate parenting ; it has shown that it is a developmental disorder of brain function that shares characteristics with others such as developmental language disorders, dyslexia, attention deficit disorder, Tourette syndrome, and others.

In 10-30% of cases there is an identifiable etiology. Some of the frequently quoted causes are herpes simplex encephalitis which involves one or both temporal lobes, intra-uterine cytomegalic or rubella infections, intra-uterine exposure to thalidomide or valproate, chromosomal anomalies like fragile-X or Angelman syndromes, genetic disorders like inadequately treated phenylketonuria, tuberous sclerosis or Cornelia de Lange syndrome, and many others (*Gillberg and*

Coleman ,1996). In the majority of cases ,there is no obvious cause (**Cohen and Volkmar, 1997).**

The causes of autism is multifactorial (**Dalton et al., 2004).** Progress in understanding the cause, let alone developing new approaches to treatment. Research on the pathogenesis of PDD has focused almost exclusively on autism (**Sophie et al., 2002).**

1-Genetic causes :

Autism is a complex largely genetic psychiatric disorder. The neuroanatomic findings in monozygotic twin pairs with autism support the role of genetic liability in autism (**Kates et al. ,2004).**

The recurrence risk for autism after the birth of an autistic child is 60 to 150 times more than the population base rate. Epidemiologically based same gender twin studies have reported higher concordance rates for autism among identical twins than among non identical twins. The mode of genetic transmission is unclear. The marked fall-off in rates of autism that occurs from identical to non identical twins or siblings suggests that a small number of interacting genes rather than one single gene is involved, with estimates of genes involved ranging from 2 to 20 (**Cook, 2001).**

These disease genes have been found to affect different steps of cortical development, including proliferation of neuronal progenitor cells, neuronal migration and maintaining

integrity of the pial surface. In many cases, syndromes with similar clinical phenotypes are caused by genes with related biochemical functions (*Mochida and Walsh, 2004*). Several full genome searches for susceptibility loci in autism using affected sibling pairs have been performed, as have a dozen candidate genes studies. Although several areas of the genome (i.e, on chromosome 7q, 1, 2, 6, 13 and 16) have been identified as regions of interest, currently no specific variation in a specific gene has been firmly established as a susceptibility gene for autism (*Cook, 2001*). The most promising may be the findings of deletions and duplications in chromosome 15 (*Dalton et al., 2004*) affecting the transport of the neurotransmitter serotonin (*Cook et al., 1997*) but many other chromosomal loci are also being considered (*Gillberg, 1998*).

Metallothionein (MT) dysfunction:

This hypothesis was proposed by William Walsh, who took extensive biochemical analyses of over 500 autistic patients and discovered that almost universally, these children have abnormal copper/zinc ratio with high body copper and low body zinc. He also discovered that the body control mechanism for copper and zinc is a function of a family of proteins called metallothionein (MT). Other functions of MT in the body include development of brain neurons, detoxification of heavy metals, maturation of the gastrointestinal tract (GIT), antioxidation, boosting immune function and delivery of zinc to cells. Because MT synthesis is enhanced by estrogen and

progesterone, it would explain the male sex predominance (4:1) seen in autism. MT dysfunction could be caused by a genetic MT defect, a genetic disorder that disables MT, or an environmental insult that disables MT (*Jepson, 2002*).

2-Mitochondrial causes

A likely etiological possibility in autism may involve a mitochondrial dysfunction with concomitant defects in neural oxidative phosphorylation within the central nervous system (*Lombard, 1998*).

Autism may be a disorder of fatty acid metabolism due to possible dysfunction of mitochondrial long chain acyl CoA dehydrogenase enzyme responsible for the beta oxidation of polyunsaturated fatty acids in the mitochondria (*Clark-Taylor and Clark-Taylor, 2004*).

This hypothesis is supported by a frequent association of lactic acidosis and carnitine deficiency in autistic patients. Also because the mitochondria are vulnerable to wide array of endogenous and exogenous factors which appear to be linked by excessive nitric acid production (*Lombard, 1998*).

A recent study revealed a strong linkage and association of autism with two single nucleotide polymorphisms (SNPs). Both were within SLC25A12 gene which is a gene encoding the mitochondrial Aspartate/Glutamate carrier (AGC1) (*Ramoz et al., 2004*).