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Study of Serum Level of L-Carnitine in Children with Non-Syndromic Autism Spectrum Disorder: A Biological Metabolic Marker

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Abstract

***Background*: Autism Spectrum Disease (ASD) is a complex developmental disorder. Children with autism spectrum disorder have low serum levels of L-carnitine. *Aim:* We aimed at assessing the reliability of using the serum level of L-carnitine as a biomarker in children with ASD. *Subjects and Methods*: This is a case-control study conducted on 60 children at the outpatient Neurobehavioral Clinic of Alexandria University Children's Hospital (AUCH), divided into 2 groups. Group 1 (research group): 30 children diagnosed with ASD and Group 2 (control group): 30 healthy children. Children of both groups were subjected to history taking, clinical neurological examination, Childhood autism rating scale (CARS), and serum carnitine level measurement. *Results:* The mean serum level of L-carnitine in children with ASD was significantly lower in cases than in control children. No statistically significant correlation between low levels of serum L-carnitine and gender was detected in the patients' group. The severity of ASD symptoms had a non-significant correlation with levels of L-carnitine. *Conclusion*: Serum L-carnitine can be considered a biochemical metabolic marker for mitochondrial dysfunction in children with ASD.**

Keywords: CARS; Biological marker; ASD; mitochondrial dysfunction; repetitive behavior

Introduction

Autism spectrum disorder (ASD) is a "complex developmental disease characterized by persistent social interaction challenges, speech and non-verbal communication impairments, and repetitive behavior constraints"[(1)]. These symptoms first occur in childhood and interfere with daily activities[(2)]. Autism affects over a million people in the United States, with millions more worldwide[(3)]. It is becoming clear that ASD is most likely caused by genetic predisposition in combination with environmental risk factors[(4)]. ASD is defined by persistent social difficulties, as well as repetitive, restricted behaviors and atypical sensory behavior, all of which are considered diag-

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nostic criteria of ASD. These indications are frequently present by the age of three, while they may not be fully developed until school age or later, and another research suggests that signals can arise between the ages of six and eighteen months. Children with severe disabilities are more likely than children with mild disabilities to be identified and diagnosed at a younger age[5]. For the diagnosis of children with ASD, a complete assessment by a specialized team with experience in ASD diagnosis and management is essential. A complete history, physical examination, neurological examination, and direct assessment of the child's language, social, and cognitive development, as well as several standardized checklists, assessment instruments, and criteria used to reach an ASD diagnosis, should all be included in the evaluation[6]. While ASD has no cure, there are number of particular interventions that can help with communication, social, and intellectual development. According to previous research, skill building and behavioral modification services can considerably improve a child's development, and these programs should begin before the age of three[7]. Therapy is given to help the youngster communicate, walk, and engage with others. The main target of treatment is to diminish related inadequacies and family hardship, as well as to improve ASD children's quality of life and daily life activities independence[8]. Multiple studies have discovered mitochondrial dysfunction (MD) in ASD patients' CNS and peripheral cells. Some autism individuals have been found to have a deficiency in carnitine[9]. Carnitine is required for poly unsaturated fatty acids (PUFA) transport through the inner mitochondrial membrane, which is necessary for beta-oxidation[10]. Acetyl carnitine is also essential for the synaptic transmission of a variety of neurotransmitters. As a result, it is necessary for mitochondrial function. Sufficient amounts of PUFAs and carnitine are also required for brain energy[11]. The link between L-carnitine levels and ASD is based on three key observations: evidence of mitochondrial failure in ASD patients, genetic elements of L-carnitine metabolism connected to autism, and the relationship between ASD severity and measured L-carnitine levels[12]. In the present study, we aimed at assessing the reliability of using the serum level of L-carnitine as a biomarker in children with ASD.

Subjects and Methods

All the children included in the study, both cases or controls, were identified and selected from the Neurobehavioural Outpatient Clinic at Alexandria University Children Hospital (AUCH). This is a case control study which was conducted on 60 children divided into two groups; (group 1): 30 children diagnosed with ASD, diagnosed on the basis of DSM-5 criteria and aged from 2 years to 6 years**.** Both sex were included[1], (Group 2) another 30 apparently normal age and sex matched children. Exclusion criteria**:** Children with specific syndromes such as fragile x syndrome and tuberous sclerosis to make sure that the results are accurate and specific to ASD not due to any other factors. Also, children with chronic diseases that might impair mitochondrial function or nutritional status were also excluded for example, epilepsy, hepatic diseases, inborn errors of metabolism,

renal diseases, cardiac diseases and malnutrition disorders. They all were subjected to the following: detailed history taking, with a focus on perinatal, developmental, and family history. A complete clinical examination was performed, with an emphasis on the neurological examination. CARS (Childhood Autism Rating Scale)[13] which is a tool that can be used with children as early as two years old to aid in the diagnosis of autism; it took 20-30 minutes to complete. It assigns a severity rating to the symptoms. The range of scores is 15 to 60. Mild to moderate autism is defined as a score of 30-36.5, whereas severe autism is defined as a score of 37-60. Laboratory investigations were performed using sandwich enzyme linked immune sorbent assay (ELISA) method to detect l-carnitine level in the whole blood. The colour of the stop solution changes from blue to yellow, and the intensity of the colour is measured using a spectrophotometer at 450nm.

Ethical considerations

The study protocol was approved by the ethical committee of Faculty of Medicine, University of Alexandria. The caregivers were asked to provide written consent for their children to take part in the study, after explaining the purpose of the study. All data and information from the participants were kept confidential**.** FAW NO:000-18699. IRB NO: 00012098

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0[14]. (Armnok, NY: IBM corp) qualitative data were described using number and percent. The Kolmogorov-Smirnov test was employed to ensure that the distribution was normal. Range (minimum and maximum), mean, standard deviation, and median were used to characterize quantitative data. The significance of the acquired results was assessed at a 5% level. For statistical significance, a 0.05 level was utilized as the cutoff value.

Results

Concerning the demographic data (age, gender, residency, and level of parents' education), there was non-statistically significant difference between the two groups regarding age of the parents' education (*p*= 0.387, 1.00 respectively). Regarding the age and place of residency, there was a male gender predominance and more children from urban areas residency among children with ASD compared to control group (*p*=0.045, 0.020 respectively) (Table 1). There was non-statistically significant difference between the two studied groups in terms of history of (antenatal events, mode of delivery, gestational age at birth, postnatal events, consanguinity, and similar condition in the family), (*p*=0.339, 0.317, 0.706, 0.418, 0.519, 0.095 respectively) (Table 2). Concerning the classification of autism severity measured by the Childhood Autism Rating scale (CARS) in cases group, 80% were classified as severe ASD. The percentages of mild and moderate ASD were equal at (10%) (Table 3). There was a strong negative relation between the levels of plasma L-carnitine in the cases compared to controls, the level of L-carnitine among children with ASD was significantly lower than the con-

trol group ($p < 0.001$). This was statistically significant (Table 4).

Table 1: The Demographic Data of both Groups				
	Children with ASD (n = 30) No. (%)	**Healthy children** (n = 30) No. (%)	**Test of sig.**	***p***
Age (months)				
Min. – Max.	26.0 – 62.0	30.0 – 55.0	t=0.873	0.387
Mean ± SD.	43.50 ± 10.43	41.53 ± 6.59		
Gender				
Male	25 (83.3%)	18 (60.0%)	χ^2=4.022*	0.045*
Female	5 (16.7%)	12 (40.0%)		
Residency				
Rural	9 (30.0%)	2 (6.7%)	χ^2=5.455*	0.020*
Urban	21 (70.0%)	28 (93.3)		
Parental education				
Illiterate	3 (10.0%)	2 (6.7%)	χ^2=0.218	FEp=1.000
Educated	27 (90.0%)	28 (93.3%)		

*χ^2: Chi square test, FE: Fisher Exact test, t: Student t-test, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05*

Table 2: Compares the perinatal and family history between children with autism and apparently normal children						
	Cases (n = 30)		**Control** (n = 30)		**χ^2**	***P***
	No.	**%**	**No.**	**%**		
Antenatal history						
Uneventful history	17	56.7	22	73.3	2.151	MCp= 0.339
Presence of diabetes mellitus	8	26.7	6	20.0		
Presence of hypertension	5	16.7	2	6.7		
Delivery						
Normal vaginal delivery	7	23.3	4	13.3	1.002	0.317
Cesarean section	23	76.7	26	86.7		
Gestational age at birth						
Full term	25	83.3	27	90.0	0.577	FEp= 0.706
Preterm	5	16.7	3	10.0		
Postnatal history						
Negative	15	50.0	20	66.7	1.996	MCp= 0.418
Positive for NIHB?	13	43.3	8	26.7		
Positive for respiratory distress	2	6.7	2	6.7		
Consanguinity						
Non consanguineous	25	83.3	23	76.7	0.417	0.519
Consanguineous	5	16.7	7	23.3		
Similar conditions in family						
Negative	22	73.3	27	90.0	2.783	0.095
Positive	8	26.7	3	10.0		

*χ^2: Chi square test, MC: Monte Carlo, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05*

Concerning the level of l-carnitine among children with ASD, there was non-statistically significant difference between males and females (p=0.296) (Table 5). The mean L-carnitine level in children with moderate and severe

ASD was (26± 4.73, and 26. ± 7.30 respectively), this was statistically insignificant (*p*=0.955) (Table 6). There was non-statistically significant relation between the L-carnitine level in children with ASD with different autism severity as classified by CARS (mild, moderate, severe), (*p*=0.800) (Table 7).

Table 3: Distribution of the studied cases according to the autism severity measured by CARS (n = 30)

	No.	%
CARS		
Mild or risky-ASD (15-29)	3	10.0
Moderate (30 - 35)	3	10.0
Severe (36 – 60)	24	80.0
Min. – Max.	15.0 – 49.0	
Mean ± SD.	39.93 ± 7.93	
Severity		
Mild or risky	3	10.0
Moderate	3	10.0
Severe	24	80.0

Table 4: Comparison between the two studied groups according to L-carnitine level

L-Carnitine level	Cases (n = 30)	Control (n = 30)	T	*P*
Min. – Max.	16.0 – 40.0	25.0 – 53.0	4.845*	<0.001*
Mean ± SD.	26.27 ± 6.91	34.70 ± 6.57		

t: Student t-test, p: p value for comparing between the studied groups,
*: Statistically significant at p ≤ 0.05

There was strong significant relation between the reduced level of L-carnitine and social abnormalities as a first observed symptom compared to other symptoms (behavioral abnormalities and language defects) among the cases group (*p*= 0.029) (Table 8).

Discussion

ASD is currently diagnosed using DSM-5-based criteria and a psychometric evaluation. Because symptoms are so difficult to diagnose, researchers are concentrating their efforts on identifying biological markers in genetics, brain imaging, and biochemical markers in the hopes of making a more accurate diagnosis[15]. Male to female ratio in ASD children was 4.5:1. Male predominance has been repeatedly reported in numerous researches[16]. Male predominance in ASD has received a lot of attention. In a similar study, Jacquemont et al.[17] proposed that females with ASD are spared from some of the disorder's symptoms, which they called the "female protective effect" (FBE). Males with ASD have more negative copy number variations (CNVs) and single nucleotide variants (SNVs) than girls with ASD. Therefore, the symptoms of ASD require greater genetic modification in the female brain than that in male brains[17]. This differs from a research by Rynkiewiez et al.[18] who argued that female underdiagnosis could be related to differences in symptom presentation, a phenomenon known

as the "female camouflage effect." Males are more likely to develop symptoms that necessitate a clinical evaluation, such as hyperactivity and violence. Clinicians, on the other hand, believe that girls with an IQ above 70 are more social, allowing the symptoms of autism to be misinterpreted and an accurate diagnosis to be delayed.

Table 5: Relation between gender and L- carnitine level in cases group

L-Carnitine level	Gender		T	*P*
	Males (n = 25)	**Females** (n = 5)		
Min. – Max.	16.0 – 40.0	25.0 – 32.0	1.077	0.296
Mean ± SD.	25.92 ± 7.47	28.0 ± 2.74		

t: Student t-test, p: p value for comparing between males and females

Table 6: Relation between CARS and L Carnitine level in cases group

L Carnitine level	CARS		t	*P*
	Moderate (30 - 35) (n = 3)	**Severe (36 – 60)** (n = 24)		
Min. – Max.	21.0 – 30.0	16.0 – 40.0	0.057	0.955
Mean ± SD.	26.33 ± 4.73	26.58 ± 7.30		

t: Student t-test , p: p value for comparing between the studied categories

Table (7): Relation between Severity and L Carnitine level in cases group (n=30)

L Carnitine level	Severity			F	*P*
	Mild or risky (n = 3)	**Moderate** (n = 3)	**Severe** (n = 24)		
Min. – Max.	16.0 – 29.0	21.0 – 30.0	16.0 – 40.0	0.225	0.800
Mean ± SD.	23.67 ± 6.81	26.33 ± 4.73	26.58 ± 7.30		

Regarding the place of residency, there was predominance to urban areas residency among children with ASD compared to control group. Mandell et al. and Fountain et al.[19, 20] both reported that children born in cities have a higher likelihood of being diagnosed with ASD. In addition, William et al.[21] established a relationship between a child's urban living and ASD. This is due to a lack of health-care services that are both accessible and available in the rural regions. Also, Palmer et al.[22] and Strachan et al.[23] stated that metropolitan areas have a higher frequency of ASD than rural locations. Due to the hygiene hypothesis, living in rural regions has protective effects due to allergen exposure such as farm animal contact, which boosts the immune system. In the present study, 83.3 percent of autistic children were born full term, and 90% of control children were delivered full term. Schieve et al.[24] showed that preterm did not explain for a significant fraction of the rise in ASD occurrence, which is similar to our findings. Several investigations of ASD have found that preterm children have a higher risk of developing the disor-

der. The link between preterm and ASD could be explained by prenatal and postnatal neurodevelopmental damage, which could result in growth and brain development problems[25].

Table 8: Relation between first observed symptom and L Carnitine level in cases group (n=30)

L-Carnitine level	First observed symptom			F	P
	Social abnormalities (n = 14)	Language defect (n = 7)	Behavioral abnormalities (n = 9)		
Min. – Max.	16.0 – 32.0	18.0 – 40.0	19.0 – 39.0	4.069*	0.029*
Mean ± SD.	23.07 ± 5.43	27.0 ± 7.96	30.67 ± 6.14		

The ASD children in this study were initially diagnosed between the ages of 26 and 62 months. Similarly, De Giacomo, stated that the average age of diagnosis was 24 months[26]. El-Baz et al. also reported that 46% of children presented at the age of 18 months, and 32% at the age of 24 months[27]. In contrast to the current study, Simekv and Koroglu[28] found that 40.3 percent of children with autism were identified at the age of three, 36.1 percent at the age of four, and 16.7 percent at the age of two. Many studies, like as Ozonoff's[29], have categorized patients with ASD according to their onset pattern. Early onset (symptoms show during the first year or so of life) and regressive autism are the two forms of autism (normal development over the first 15-19 months of life, followed by a loss of skills that correlates with the onset of autism). These findings correlate with Gray and Tonge[30], who discovered that parents become concerned about autistic behavior when their children are 12–30 months old. In regard to the L- carnitine level, the current work found that autistic children showed significantly lower serum levels of l-carnitine compared to controls. In consistence with the present work, Fillpek et al,[31] studied The levels of total and free carnitine in control and ASD patients and found that autistic youngsters had lower levels of total and free carnitine and above 80% of patients with autism have L carnitine levels below the reference range. In present study, there was male predominance (male to female ratio was 5:1); however, there was non-significant difference regarding the serum levels of L-carnitine between both genders. This is in partial agreement with Baxter et al, 2005[32] who suggested that Males are around 4.5 times as likely than females to be affected by ASD. Also, Christensen 2016[33] suggested the same result. The CARS was used to assess the severity of autism symptoms[34]. In the current study, there was no statistically significant relation between the L-carnitine level in autistic patients with different autism severity scores, however, there was strong relation between the reduced level of L-carnitine and social impairment as a first observed core symptom compared to other symptoms (behavioral abnormalities and language defects). This could be explained by the sample selected, 80% of children were rated as severe autism while only 20% were rated as risky and moderate ASD. This is different from

Fahmy et al.[35] who studied the effect of L- carnitine supplement on the severity of symptoms of ASD, they reported significant improvement in the behavior aspect of autism and decreased severity assessed by CARS.

Conclusion

Serum L- carnitine can be considered as a biochemical metabolic marker for mitochondrial dysfunction in children with ASD.

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