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Original Research Article

Investigation of Carnitine Levels in some Biological Fluids

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ABSTRACT

Autism spectrum disorder (ASD) refers to a certain degree of neurodevelopmental disorder in children that usually occurs during the first years of life. Until now, the causes and treatments for autism have remained obscure. Research has shown that ASD has been linked to a genetic defect in carnitine metabolism. This study was aimed at evaluating the levels of carnitine in the saliva and urine of children with ASD towards proposing a diagnostic biomarker. Sixty children ranging in age from 2 to 13 years were recruited for the study. The subjects were grouped into ASD and healthy control groups. Saliva and urine were collected from both groups. The enzyme-linked immunosorbent assay (ELISA) technique was used to estimate the total carnitine in the saliva and blood samples of the autistic children and the healthy control group. The results of the study showed that there was no significant difference in the level of salivary carnitine between the children with ASD (2.22±0.37 nmol/ml) and the normal children (2.33±0.86 nmol/ml). Meanwhile, there was a significantly lower average amount of carnitine in the urine of the ASD group (28.61±8.16 nmol/ml) compared to the control group (36.13±13.12 nmol/ml). A cut-off value of < 30.23 nmol/ml was obtained with a sensitivity level of 76.7 % and a specificity level of 66.7 %, with an overall accuracy level of 70.1 %.The findings of the study suggest that low urinary carnitine is a strong predictor of autism, and a cut-off value of < 30.23 nmol/ml provided a reliable diagnostic tool.

Keywords: ASD, Autism, Carnitine, Saliva, Urine

Introduction

Carnitine is a biomarker of mitochondrial function and has an active role in lipid metabolism in cells. About 5% of children with autism have mitochondrial problems.[1] In autism, markers such as creatine kinase, ammonia, and aspartate aminotransferase are frequently employed as signs of mitochondrial dysfunction. However, several studies have revealed that novel indicators of mitochondrial dysfunction, such as lactate dehydrogenase, lactate oxidase, pyruvate kinase, hexokinase, calcium, and potassium, can predict the occurrence of any of these specific abnormal biomarkers in autistic subjects. This could potentially predict the presence of any of these aberrant biomarkers in autistic individuals.[2-4] A genetic abnormality in carnitine metabolism has been related to autism spectrum disease (ASD), and a primary carnitine deficit was reported to induce ASD in one case report. Carnitine supplementation has been shown to improve symptoms in patients with ASD in clinical studies. Since then, the role of carnitine in ASD has been recognized, and its absence has been established as a biomarker for a metabolic subtype of ASD.[3]

L-carnitine is a generic name for a quaternary ammonium chemical compound that has an important participation in the metabolic process in plants and most mammals, as well as some types of microorganisms.[5] Carnitine is required for energy metabolism because it transports long-chain fatty acids from the cytosol to the mitochondria, where they are oxidized for energy production and the elimination of metabolic waste from/within the cells.[6]

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Also, it is an amino acid that is spontaneously produced in the kidneys and liver from two necessary amino acids: lysine, and methionine. The body stores carnitine in the brain, heart, liver, and sperm, as well as in skeletal muscle, and carnitine is abundant in animal products and low in plant products.[7] The body tends to absorb more carnitine from food than it does from supplements. Studies show that the body's absorption of L-carnitine through food is approximately 54-86%, while this percentage is significantly reduced in the body's absorption of nutritional supplements, as it does not exceed 14-18%. Despite this difference arising from malabsorption, intake of carnitine through supplementation remains higher than food.[8]

Excess carnitine in the serum is excreted by the kidneys, where it is filtered and excreted with urine out of the body. Specific concentrations of acyl-carnitine and L-carnitine are being investigated in the urine of people with metabolic disorders within the mitochondria, a condition of defective organic acyl acid compounds. It includes propionic acid, hypo carboxylase, isovaleric acid, methylmalonic acid, 3-hydroxy-3-methyl glutaric acid, glutaric acid, CoA dehydrogenase, and others.[9] Carnitine has an important role in the central nervous system, and this role is demonstrated in the mitochondrial metabolism of fatty acids. In one study, abnormal fatty acid metabolism was documented in people with autism. The study recommended that autistic people should be accurately classified as a clinical category. Because of the nature of autism spectrum disorder, it can include people with intellectual disabilities, epilepsy, and even hereditary Mendelian conditions.[10-13]

The aim of this study was to evaluate the levels of carnitine in the various body fluids (saliva and urine) of children with autism.

Materials and Methods

Ethical clearance

The study was approved by the Ethical Committee of the University of Al-Qadisiya, Iraq. The study was registered by the Scientific Committee in the College of Medicine at the University of Al-Qadisiya (CMUQ3544 on 15/12/2020).

Equipment used

In this study, the equipment used includes a 40ºC deep freezer (Froilabo, France), a washer for ELISA analysis, an incubator, a timer, 1 ml Ependroff tubes, sterile wide saliva collection tubes, sterile urine collection tubes, gloves, and medical masks, and total human carnitine (ELISA Kit, Bioassay).

Sample collection and experimental grouping

Saliva and urine were collected from 60 children of both sexes, who were divided into two groups (the ASD and control groups). The ASD group consisted of 30 autistic children (19 males and 11 females), aged 2–13 years. Urine and saliva samples were collected and sent to the departmental laboratory of Al-Diwaniyah Teaching Hospital, Al-Diwaniyah, Iraq. The samples were kept in a cool box and placed in a special refrigerator at -40ºC. Thirty healthy children were selected as a control group (20 males and 10 females), ranging in age from 3 to 13 years. The controls were not relatives of the patients and did not show symptoms of psychiatric or neurological disorders. They also did not have a family history of autism spectrum disorder or other neurological disorders. Urine and saliva samples were collected from each individual, and the sampling period was from October 2020 to January 2021. All participants were unrelated Iraqi children with similar geographic and socio-demographic data. All the children's relatives gave their written informed consent to participate in the study and to allow urine and saliva samples to be collected for the study. The autistic children who participated in this study were diagnosed by psychiatrists in government hospitals and private clinics.

Evaluation of total carnitine

Total carnitine was measured using the enzyme-linked immunosorbent assay (ELISA) technique. Test materials from the Bioassay Technology Laboratory (UK) were used. All the experimental steps were performed at room temperature. One hundred and twenty litre of the standard (320 nmol/ml) was mixed with 120 µL of the standard dilute solution to make a standard stock solution with a concentration of 160 nmol/ml. The resulting solution was gently stirred for 15 minutes before further dilutions were made. Multiple standard points were prepared by serial dilution in a 1:2 ratio with the standard stock (160 nmol/ml), to obtain 10, 20, 40, and 80 nmol/ml as illustrated in Figure 1. It was then diluted to zero (0 nmol/ml), which served as a standard diluent.

Statistical analysis

Microsoft Office Excel 2010 and SPSS version 23 were used to collect, analyze, and present the data. The Kolmogorov-Smirnov test was performed to assess if the numeric variables were normally distributed, and if they were, the mean (an index of central tendency), the standard deviation (an index of dispersion), and the median (an index of central tendency) were employed to express them. The numeric variables that were not normally distributed were expressed as the median (indexes of central tendency and dispersion).

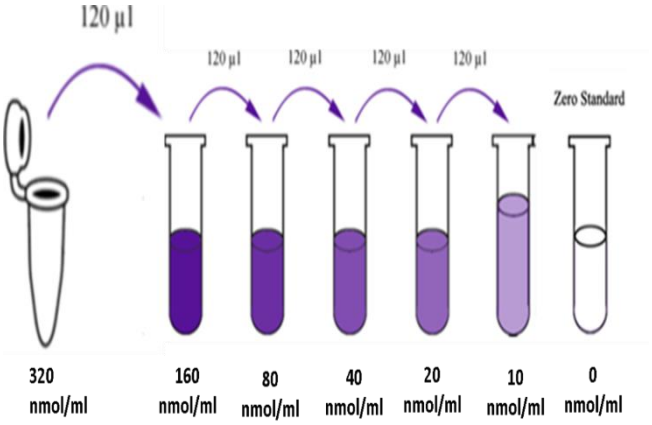

Figure 1: The experimental procedure used to make the serial dilution

Results and Discussion

The comparison of mean salivary and urine carnitine between the study and control groups is presented in Table 1 and Figure 2. The mean salivary carnitine level in ASD was 2.22±0.37 nmol/ml, compared to 2.33±0.86 nmol/ml in the control group. There was no significant difference in the salivary carnitine in the ASD group compared to the control group (p = 0.523). On the other hand, the mean urine carnitine of ASD in the study group was significantly (p = 0.010) lower than that of the control group, with values of 28.61±8.16 and 36.13±13.12 nmol/ml, respectively (Figure 3 and Table 1). The results of the Receiver operator characteristic (ROC) analysis performed to find the cut-off value of urine carnitine that could predict a diagnosis of ASD are shown in Table 2 and Figure 4. Accordingly, a cut-off value of < 30.23 nmol/ml was identified with a sensitivity level of 76.7 % and a specificity level of 66.7 %, with an overall accuracy level of 70.1 %. The area under the curve (AUC) was more than 0.7; therefore, the cut-off value can be adopted with acceptable accuracy to predict a diagnosis of ASD.

Carnitine is particularly susceptible to toxicity caused by heavy metals, environmental contaminants, and/or bacteria, and research indicates that children with autism have altered microbiomes and metabolomes, reducing the ability of the carnitine shuttle to function effectively as a result of microbial by-products.[1] According to Ratajczak and colleagues, the level of salivary carnitine in autistic individuals is significantly lower than that of control subjects, but the present investigation found no significant difference in salivary carnitine in the ASD group compared to the healthy group. As a result, carnitine deficiency in autistic saliva could be the explanation, although the difference in the present study did not achieve statistical significance, possibly because youngsters were included.[14] Therefore, other than the information provided by Ratajczak in 2015, there is no additional published information about the role of salivary carnitine.

Carnitine is particularly susceptible to toxicity caused by heavy metals, environmental pollutants, and/or bacteria. Research suggests that children with autism have altered microbiomes and metabolites, impairing the ability of the carnitine shuttle to function effectively as a result of microbial byproducts.[15,16] Ratajczak referenced his study, which found that autistic individuals' salivary carnitine levels were much lower than that of controls, but that there was no significant difference in salivary carnitine in the ASD group compared to the healthy group. Hyposalivation in autism is the explanation for maybe-carnitine, but the difference in this study did not achieve statistical significance, probably because children were included in the study and did not address their teens or adults. As a result, it can be concluded that there is no more published information about the role of salivary carnitine other than that mentioned by Ratajczak in 2015.[15]

L-carnitine, specifically mitochondrial fatty acid metabolism, is required for central nervous system function. Autism spectrum disorder individuals have been found to have impaired fatty acid metabolism. The disorder is a neurodevelopmental disorder that is most often discovered in children under the age of five. Patients with ASD need to be classified carefully since this clinical group includes patients with an intellectual disability or high functioning, seizures, language difficulties, or linked Mendelian genetic diseases. Acyl groups are provided by L-carnitine, which enhances the brain's production of acetylcholine, which in turn increases the expression of the growth-associated protein-43, protects cells from apoptosis and neuronal damage, and stimulates the transmission of nerve signals.[15,17,18] Analysis of acylcarnitines in a dried blood spot may be useful in the diagnosis and treatment of ASD. The acylcarnitine profiles of children with ASD may reveal mitochondrial malfunction and aberrant fatty acid metabolism. In autism, a lack of L-carnitine metabolism is accompanied by other metabolic abnormalities such as the Krebs cycle and respiratory chain complex activity, both of which indicate mitochondrial dysfunction. Patients with ASD may benefit from using L-carnitine supplements to help with behavioral and cognitive symptoms.[15,19]

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Table 1: Comparison of mean salivary and urine carnitine levels between ASD and control groups

Characteristic	Control group *N* = 30	ASD group *N* = 30	*P*
Saliva carnitine concentration (nmol/ml)			
Mean ± SD	2.33 ± 0.86	2.22 ± 0.37	0.523 I
Range	1.71 - 5.08	1.71 - 3.25	NS
Urine carnitine concentration (nmol/ml)			
Mean ± SD	36.13 ± 13.12	28.61 ± 8.16	0.010 I **
Range	8.57 - 62.72	15.02 - 44.67	

N: number of cases; I: independent samples *t*-test; NS: not significant at $p > 0.05$; **: significant at $p \leq 0.01$

ASD: Autism spectrum disorder

Table 2: Characteristics of the ROC curve.

Characteristic	Value
Cutoff	> 30.23 mg/dl
AUC (95 % CI)	0.701 (0.560 to 0.805)
Accuracy %	70.10 %
Sensitivity %	76.7
Specificity %	66.7
P-value	0.006 **

AUC: Area under the curve; CI: Confidence interval; **: Significant at $p \leq 0.01$; Receptor factor characteristics (ROC) were used to find the best cut-off for urinary carnitine (in terms of accuracy, sensitivity, and specificity) to predict a diagnosis of autism.

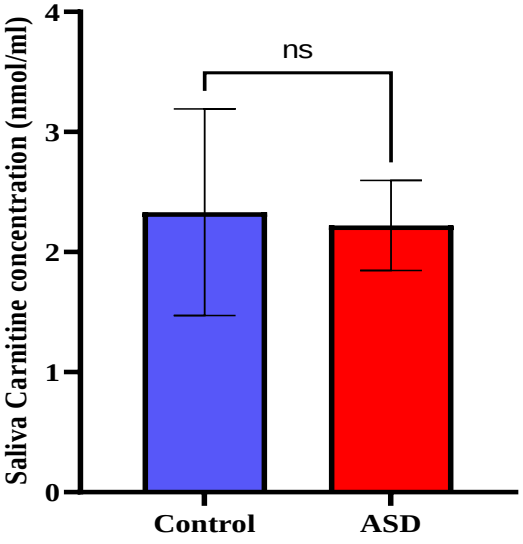

Figure 2: Comparison of saliva carnitine levels between the ASD and the control groups. Data were presented as mean ± SD; ns: Not significant at p > 0.05; ASD: Autism spectrum disorder.

Conclusion

The findings of this study reveal that low urinary carnitine might be used as a predictor of autism. The originality of this study is that a urine carnitine cut-off value of 30.23 mg/dl that can accurately predict an ASD diagnosis with a 70.1% accuracy rate was discovered. The study provides an easy non-invasive technique for screening autism in general population or for follow-up of the therapy.

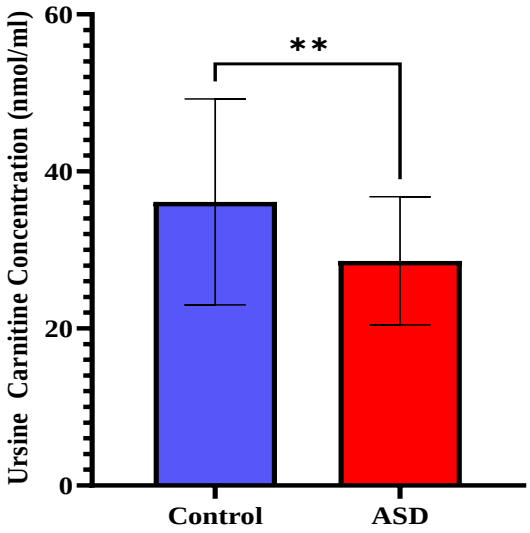

Figure 3: Comparison of urine carnitine levels between the ASD and the control groups.
Data were presented as mean ± SD; **: Significant at p ≤ 0.001; ASD: Autism spectrum disorder.

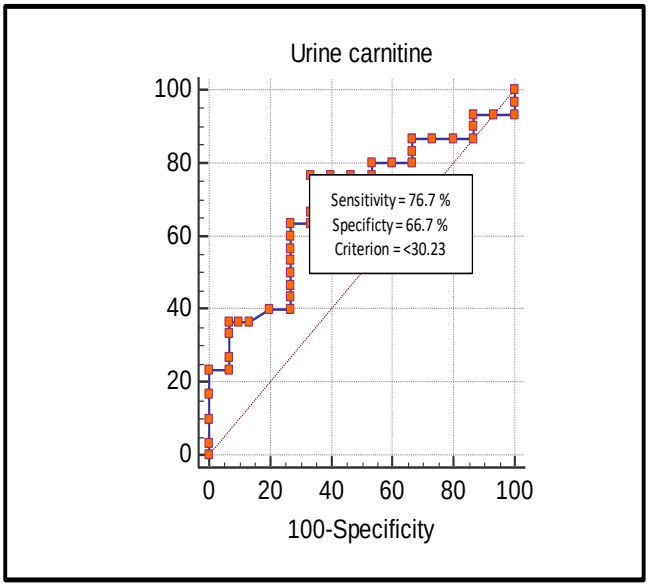

Figure 4: Demonstration of a receiver operator characteristic (ROC) curve analysis to predict an autism diagnosis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. MacFabe D. Autism: metabolism, mitochondria, and the microbiome. Glob Adv Health Med. 2013;2(6):52-66.
2. Pinto JV, Moulin TC, Amaral OB. On the transdiagnostic nature of peripheral biomarkers in major psychiatric disorders: a systematic review. Neurosci Biobehav Rev. 2017; ;83:97-108.
3. Frye RE, Vassall S, Kaur G, Lewis C, Karim M, Rossignol D. Emerging biomarkers in autism spectrum disorder: a systematic review. Ann Transl Med. 2019; 7(23):1-21.
4. Hollis F, Kanellopoulos AK, Bagni C. Mitochondrial dysfunction in Autism Spectrum Disorder: clinical features and perspectives. Curr Opin Neurobio. 2017; 45:178-87.
5. Cozzolino R, De Magistris L, Saggese P, Stocchero M, Martignetti A, Di Stasio M, Malorni A, Marotta R, Boscaino F, Malorni L. Use of solid-phase microextraction coupled to gas chromatography–mass spectrometry for determination of urinary volatile organic compounds in autistic children compared with healthy controls. Analy Bioanalytical Chem. 2014; 406(19):4649-62.
6. Longo N, Frigeni M, Pasquali M. Carnitine transport and fatty acid oxidation. Biochimica et Biophysica Acta (BBA)-Mol Cell Res. 2016; 1863(10):2422-35.
7. Esterhuizen K, Van der Westhuizen FH, Louw R. Metabolomics of mitochondrial disease. Mitochondrion. 2017; 35:97-110.
8. Gupta A, Rawat S, Gupta P. Clinical Research and Therapeutic Importance of Dietary Supplement L-Carnitine. Asian J Pharmac Res. 2018; 8(1):47-58.
9. El-Gharbawy A, Vockley J. Inborn errors of metabolism with myopathy: defects of fatty acid oxidation and the carnitine shuttle system. Pediatric Clin. 2018; 65(2): 317-35.
10. Fahmy SF, El-Hamamsy MH, Zaki OK, Badary OA. l-Carnitine supplementation improves the behavioral symptoms in autistic children. Res Autism Spectr Disord. 2013; 7(1):159-66.
11. Hameedi AJ, Saud AM. Association of the Intronic Polymorphism rs3773364 A> G in Synapsin-2 Gene with Epilepsy Patients in Iraq. Iraqi J Sci. 2021: 2169-75.
12. Bölte S, Golan O, Goodwin MS, Zwaigenbaum L. What can innovative technologies do for autism spectrum disorders?. Autism. 2010; 14(3):155-9.
13. Strickland CM, Drislane LE, Lucy M, Krueger RF, Patrick CJ. Characterizing psychopathy using DSM-5 personality traits. Assessment. 2013; 20(3):327-38.
14. Ngounou Wetie AG, Wormwood KL, Russell S, Ryan JP, Darie CC, Woods AG. A pilot proteomic analysis of salivary biomarkers in autism spectrum disorder. Autism Res. 2015; 8(3):338-50.
15. Mamedov I, Zolkina I, Nikolaeva E, Glagovsky P, Sukhorukov V. Carnitine insufficiency in children with inborn errors of metabolism: prevalence and treatment efficacy. J Pediatr Endocrinol Metab. 2015; 28(11-12):1299-304.
16. Smith PA. The tantalizing links between gut microbes and the brain. Nature. 2015; 526(7573):312-4.
17. Marcovina SM, Sirtori C, Peracino A, Gheorghiade M, Borum P, Remuzzi G, Ardehali H. Translating the basic knowledge of mitochondrial functions to metabolic therapy: role of L-carnitine. Trans res. 2013; 161(2):73-84.
18. Karlic H, Lohninger A. Supplementation of L-carnitine in athletes: does it make sense?. Nutrition. 2004; 20(7-8):709-15.
19. Soliman NA, EL-Gaafary MN, Shalaby AA, Hussein OM, Taha NM. Physiological and Histopathological Effects of Two Seed Extracts (Phoenix dactylifera and Raphanus sativus) and L-Carnitine Drug on Semen Quality and Testicular Sexual Hormonal Changes in Adult Male Rabbits. Trop J Nat Prod Res. 2021; 5(10):1709-1715.