Original Article

Study of Biochemical Changes in Children with Autism Spectrum Disorder Aged 3-13 Years in Thi-Qarcetnter of Autism

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ABSTRACT

Objective: To find out the causes of autism spectrum disorder therefore some biochemical parameters were measured include serum lactate, pyruvate, lactate to pyruvate ratio, lactate dehydrogenase, ferritin and glutamate.

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Thi-Qar Autistic Center, Nasiriya City, Iraq from 1st September 2022 to 28th February 2023.

Methods: This study contained 192 children among which 96 patients were diagnosed as cases of ASD and age range was 3 to 13 years. The control group contained 96 children with ages range was 3-13 years. The Enzyme Linked Immunosorbent Assay method is applied in detection of parameters.

Results: there were significant statistical association between biochemical parameters and autism spectrum disorder in compare with control group except serum pyruvate in which there was no significant association

Conclusion: The cause of autisms spectrum disorder may be a defect in mitochondrial functions where there was elevation in serum lactate and lactate to pyruvate ratio.

Key Words: Autism spectrum disorder, Lactate, Pyruvate, Lactate dehydrogenase, Ferritin

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INTRODUCTION

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that are characterized by early onset communication dysfunctions, limitations in social interaction, and repetitive and stereotyped movements with narrow interests.[1] The clinical picture of ASD often start to appear between 12 and 18 months and the diagnosis is typically made at two years of age.[2] Although the ASD is unknown etiology but it may be due to the interaction of multiple factors, include biological, environmental, and genetic factors.[3] The prevalence of ASD is approximately 1% in the pediatric age group worldwide. This prevalence has increased over time, and there are variations within and between sociodemographic groups.[4] The prevalence of ASD is about 2% in the pediatric age group in the United States.[5]

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The incidence is unaffected by race or ethnicity, and children from low socioeconomic status may delay diagnosis. The male to female ratio is about 4:1 and the recurrence rate among identical twins is high[6] and ASD is classified as a mild when individuals do not receive support, with visible disturbances in social communication while in moderate type, there is marked deficiencies in communication skills and unusual response to social activities while in severe type there are severe deficiencies in social communication skills.[7,8]

METHODS

It is a case-control study was performed at Thi-Qar Autistic Center, Nasiriya City, Iraq from 1st September 2022 to 28th February 2023. The collection of samples were performed for 192 children participate in this study included the patients group who include 96 children with ASD with ages range was 3 to 13 years. The control group consists of 96 apparently healthy children with ages range was 3-13 years. After collection of 3 ml of blood sample from all participant in serum separating gel tubes for biochemical tests and were allowed to clot for 15 minutes and then centrifuged at 1800 ×g for about 15 min, then separated into small aliquots and froze in a 20-ºC to be used for estimation of biochemical tests: serum lactate, pyruvate, ferritin, LDH, glutamate levels. The methods of measurement were enzyme linked immunosorbent assay kits from SunLong-biotech, China. This study

was approved based on the ethical principles that originated in the Declaration of Helsinki. It was conducted with patients' verbal and analytical approval before taking the sample. Children with schizophrenias, cerebral palsy and patient with iron supplement were included. The data was analyzed using Software Package for Social Science (SPSS-22.0 version). The t-test has been used to determine the significant difference between the groups. Significant difference if $p<0.05$, while very significant if $p<0.01$ and high significant if $p<0.001$.

RESULTS

There was a significant elevation in serum lactate in autistic patients subgroups (G1 and G2) in compared with control subgroups ($P<0.05$) and there were no significant difference in serum lactate and all other parameter in this study between patients sex subgroups of male and female ($p>0.05$) also between two patients age subgroups (G1 and G2) ($p>0.05$). The normal value of serum lactate is less than 22mg/dl (Table 1).

There was a no statistically significant difference between autistic patients age subgroups and apparently healthy control age subgroups in serum pyruvate ($P>0.05$), and the normal value of pediatric pyruvate is 0.3-1.5 mg/dl (Table 2). There was a significant statistically difference in serum lactate to pyruvate ratio between autistic patients age subgroups and control age subgroup ($P<0.05$) [Table 3].

There was significant statistical elevation in serum LDH in autistic patients subgroups in compare with healthy control subgroups ($P<0.01$). The normal value for LDH in 3-13 years is 120-300 U/L (Table 4).

There was a significant statistically difference in serum ferritin between patients subgroups and healthy control subgroups ($P<0.01$) and ($P<0.01$) also there were 10 cases with low serum ferritin or anemia who form 9.6% of total patients, considering the normal range of pediatric serum ferritin matching the age is 10-105 ng/ml (Table 5).

There was very significant statistically elevation of serum glutamate in autistic patients subgroups in compare with healthy control subgroup ($P<0.01$) and ($P<0.01$). The normal value of glutamate in this age group is 0.34-3.68 mg/dl (Table 6).

Table No.1: Serum lactate levels in patients and control groups

Parameter	Subgroups	No.	Mean±SD	Min.	Max.	P-value
Serum Lactate (mg/dl)	G1:Patients (3-8 yr)	72	16± 11	4	36	P<0.05
	G1:Control (3-8 yr)	72	11.7±3.8	8	20	
	G2:Patients (9-13 yr)	24	17± 8	5	35	P<0.05
	G2:Control (9-13 yr)	24	12.2 ± 4	6	18	
	Male	75	15.7± 8	4	36	p>0.05
	Female	21	17.3± 9	6	32	
	G1: Patients (3-8 yr)	72	16 ± 11	4	36	p>0.05
	G2:Patients (9-13 yr)	24	17±8	5	35	

Table No.2: Serum pyruvate levels in patients and control groups

Parameter	Subgroups	No.	Mean±SD	Min.	Max.	P-value
Serum pyruvate (mg/dl)	G1:Patients (3-8 yr)	72	1.10±0.23	1.40	0.6	P>0.05
	G1:Control (3-8 yr)	72	0.98±0.21	1.30	0.5	
	G2:Patients (9-13 yr)	24	1.20±0.23	1.35	0.4	P>0.05
	G2:Control (9-13 yr)	24	1.1±0.20	1.25	0.5	
	Male	75	1.09±0.21	1.40	0.5	p>0.05
	Female	21	1.13±0.25	1.35	0.6	
	G1: Patients (3-8 yr)	72	1.07±0.20	1.36	0.6	p>0.05
	G2:Patients (9-13 yr)	24	1.19± 0.17	1.40	0.8	

Table No.3: Serum lactate to pyruvate ratio in patients and control groups

Parameter	Subgroups	No.	Mean±SD	Min.	Max.	P-value
Serum lactate to pyruvate ratio	G1:Patients (3-8 yr)	72	14± 10	6	21	P<0.05
	G1:Control (3-8 yr)	72	11.93±3.8	7	14.6	
	G2:Patients (9-13 yr)	24	14.4± 8	4	23	P<0.05
	G2:Control (9-13 yr)	24	11.1± 4.3	5	13	
	Male	75	14.38± 8	4	30	p>0.05
	Female	21	12.61± 6	6	28	
	G1: Patients (3-8 yr)	72	14 ± 10	6	21	p>0.05
	G2:Patients (9-13 yr)	24	14.4 ± 8	4	23	

Table No.4: Serum lactate dehydrogenase in patients and control groups

Parameter	Subgroups	No.	Mean±SD	Min.	Max.	P-value
Serum lactate dehydrogenase (U/L)	G1:Patients (3-8 yr)	72	316±155	126	450	P<0.01
	G1:Control (3-8 yr)	72	254.4± 50.5	146	290	
	G2:Patients (9-13 yr)	24	304± 128	120	436	P<0.01
	G2:Control (9-13 yr)	24	240± 60	130	280	
	Male	75	311±140	140	480	P>0.05
	Female	21	314± 110	126	420	
	G1: Patients (3-8 yr)	72	316±155	146	480	p>0.05
	G2:Patients (9-13 yr)	24	304 ± 124	160	410	

Table No.5: Serum ferritin in patients and control groups

Parameter	Subgroups	No.	Mean±SD	Min.	Max.	P-value
Serum ferritin (ng/ml)	G1:Patients (3-8 yr)	72	35±20	4	100	P<0.01
	G1:Control (3-8 yr)	72	47± 21	16	102	
	G2:Patients (9-13 yr)	24	39.7±18	5	105	P<0.01
	G2:Control (9-13 yr)	24	48.8±26	14	106	
	Male	75	36 ±22	8	100	P>0.05
	Female	21	31±20	4	90	
	G1: Patients (3-8 yr)	72	35±20	4	100	p>0.05
	G2:Patients (9-13 yr)	24	39.7±18	5	96	

Table No.6: Serum glutamate in patients and control groups

Parameter	Subgroups	No.	Mean±SD	Min.	Max.	P-value
Serum glutamate (mg/dl)	G1:Patients (3-8 yr)	72	2.28 ±0.73	0.7	3.5	P<0.01
	G1:Control (3-8 yr)	72	1.55 ± 0.38	0.9	2.6	
	G2:Patients (9-13 yr)	24	2.1±0.61	0.6	3.6	P<0.01
	G2:Control (9-13 yr)	24	1.4± 0.48	0.4	2.2	
	Male	75	2.3 ± 0.77	0.8	3.5	P>0.05
	Female	21	2.1±0.90	0.7	3.3	
	G1: Patients (3-8 yr)	72	2.28± 0.73	0.7	3.5	P>0.05
	G2:Patients (9-13 yr)	24	2.1±0.61	0.6	3.6	

DISCUSSION

The possible cause of elevation of serum lactate and lactate/pyruvate ratio is one of several inherited metabolic disorder of gluconeogenesis, pyruvate oxidation, Krebs cycle or the respiratory chain disorder or production of glycolysis overwhelms the utilization of pyruvate in mitochondria[9] or harmful variants of nuclear genes encoding mitochondrial related proteins, lead to mitochondrial dysfunction.[10,11] There was a clear link between autism and mitochondrial dysfunctions which combined with biochemical alterations.[12] These results agree with most studies of Miae et al[13] and Shahjadi et al[14] in which there were high levels of serum lactate in autistic patient in compare with healthy control group and disagree with another study in which there was low serum lactate in ASD patient in compare with control group.

Pyruvate metabolism is crucial for energy homeostasis and mitochondrial fusion/fission. In this study, there was normal level of pyruvate with high level of serum lactate and high serum lactate dehydrogenase which responsible for conversion of serum pyruvate into serum lactate and this may reflect some mitochondrial dysfunction or difficulty in conversion of serum pyruvate to acetyl CoA and instead convert to serum lactate.[15] This results agree with Miae et al[13] in which there were no significant difference between patients and control groups and disagree with Giulivi C, et al[16] in which there were elevation of serum pyruvate in autistic children in compare with control groups.

The lactate to pyruvate ratio reflects the reduction-oxidation state of the cytosolic compartment and indicates the ratio of oxidized NAD^+ to reduced NADH and in patients with ASD may decrease NAD+/NADH ratio when high lactate to pyruvate ratio[17] and much higher oxidized redox state in ASD might promote anaerobic glycolytic more than oxidative phosphorylation for supply ATP.[12] These results agree with Dhillon S, et al[11] and Oliveira et al[10] in which there were a significant elevation in serum lactate to pyruvate ratio and disagree with another study in which there was no significant difference in serum lactate to pyruvate ratio between autistic subgroups and control subgroups.

Serum LDH activity has been found to be increased in ASD patients. LDH, are often used as a marker for mitochondrial dysfunction, as less pyruvate is

metabolized through the tricarboxylic acid cycle. Lactate interconvert with pyruvate by LDH.[17] One of the major pathways involve in developmental cognitive disorders is shift in energy production from mitochondrial oxidative phosphorylation to aerobic glycolysis despite the availability of oxygen.[18] These results agree with another studies conducted by a researchers, in all studies there were significant elevation in LDH level in patients group compare to control groups and there were not found any study in with low LDH in autistic patients.

Patient with ASD has been found to eat non food material from ground (pica) and eat only limited food groups, possible with low iron content and aggressive feeding behaviors. This supports iron deficiency and malnutrition in ASD. These results agree with Pakyurek M, et al[19] and Prakash P, et al[20] in studies there were significant decrease in serum ferritin in patients group and disagree with Çelik P, et al[21] and Gunes S, et al[22] in which were no significant difference in serum ferritin between patient and control groups.

CONCLUSION

The cause of autisms spectrum disorder may be related to dysfunctions of mitochondria where there was elevation in serum lactate and lactate to pyruvate ratio and high serum glutamate may be attributed to dysfunction of carrier protein gene of mitochondria wall. Low serum ferritin in patients may be related to bad food regime intake while high LDH may be response to high serum lactate.

Author's Contribution:

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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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REFERENCES

1. Montanari A, Martella G, Bonsi P, Mirangolo M. Autism spectrum disorder, focus on glutamatergic neurotransmission. Int J Mol Sci 2022;23:3861.
2. Shuang Qiu, Yingjia Qiu, Yan Li, Xianling C. Genetics of autism spectrum disorder: an umbrella review of systematic reviews and meta-analyses. Translational Psychiatr 2022;12:249.
3. AL-Ansari NAM, Ahmed EMM, Al-Maliky HAM. Analysis of Glutathione S-Transferase M1 and T1 Polymorphism in Samples of Iraqi Children with Autism. Mustansiriya Med J 2017;16(3):15-9.
4. Zidan J, Fombonne E, Scorah J, Ibrahim A, Sexena S, Yusuf A, et al. Global prevalence of autism: A systematic review update. Autism Res 2022; 15:778-790.
5. Rose S, Niyazov DM, Rossigno DA, Goldenthal M, Kahler SG, Richard E. Clinical and molecular characteristics of mitochondrial dysfunction in autism spectrum disorder. Molecular Diagn Therapy 2018; 22:571–93.
6. Kliegman RM. St Geme JW, Blum NJ, Shah SS. Nelson Textbook of Pediatrics 21st Elsever Saunders. Philadelphia;2018.p.1725–49.
7. Yücehan Y, Başak B. A systematic review of the thesis on language and communication skills of individuals with autism spectrum disorder. Elementary Educ Online 2020;19(4):2542-50.
8. Burtis CA, Ashood ER, Bruns DE. Clinical chemistry and molecular diagnostics. 5th ed. USA; 2013.p.590-91
9. Parikh S, Goldstein A, Koenig MK, Scaglia F, Gregory M, Russell et al. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med 2015;17(9): 689-701.
10. Oliveira G, Diogo L, Grazina M, Garcia P, Ataíde A, Marques C, et al. Mitochondrial dysfunctionIn autism spectrum disorders: a population-based study. Develop Med Child Neurol 2005;47:185–9.
11. Dhillon S, Hellings JA, Butler MG. Genetics and Mitochondrial Abnormalities in Autism Spectrum Disorders: A Review. Curr Genomics 2011;12(5):322-32 .
12. Nabi SU, Rehman MU, Arafah A, Taifa S, Khan IK, Khan A, et al. Treatment of autism spectrum disorders by mitochondrial-targeted drug: future of neurological diseases therapeutics. Curr Neuropharmacol 2023;21: 1042-64.
13. Miae OH, Kim SA, Yoo HJ. Higher lactate level and lactate-to-pyruvate ratio in autism spectrum disorder. Exp Neurobiol 2020;29(4):314-22.
14. Shahjadi S, Khan AS, Ahmed MU. Mitochondrial dysfunction in early diagnosed autism spectrum disorder children. J Dhaka Med Coll 2017; 26(1):43-7.
15. Kim MJ, Lee H, Chanda D, Thoudam T, Harris RA, Lee IK. The role of pyruvate metabolism in mitochondrial quality control and inflammation. Mol Cells 2023; 46(5): 259-67.
16. Giulivi C, Zhang YF, Klusek A, et al. Mitochondrial dysfunction in autism. JAMA 2010;304(21).
17. Mazón-Cabrera R, Vandormael P, Somers V. Antigenic targets of patient and maternal

autoantibodies in autism spectrum disorder. Frontiers Immunol 2019;10.

18. Vallée A, Vallée JN. Warburg effect hypothesis in autism Spectrum disorders. Vallée Vallée Molecular Brain 2018;11:1.
19. Pakyurek M, Azarang A, Iosif AM, Nordah T. Assessment of biometal profile in children with autism spectrum disorder, with attention deficit hyperactivity disorder, or with otherpsychiatric diagnoses: a comparative outpatient study. Psychopathologica 2020;4(1):6.
20. Prakash P, Kumari R, Sinha N. Kumar S, Sinha P. Evaluation of iron status in children with autism spectral disorder: a case-control study. J Clin Diagn Res 2021;15(7).
21. Çelik P, Sucak IA, Yakut HI. Iron, vitamin D and B12 levels of young children with autism spectrum disorder at diagnosis. J Dr Behcet Uz Child Hosp 2022;12(2):142.
22. Gunes S, Ekinci Q, Celik T. Iron deficiency parameters in autism spectrum disorder: clinical correlates and associated factors. Italian J Pediatr 2017; 43:86.