RESEARCH PAPER

Inborn Errors of Metabolism Associated With Autism Among Children: A Multicenter Study from Iran

HOSSEIN MORAVEJ,[1,2] SOROOR INALOO,[1] SAMAN NAHID,[3] SHOKROLLAH MAZLOUMI,[2] HAMID NEMATI,[4,5] TOKTAM MOOSAVIAN,[5] JAFAR NASIRI,[6] FARIBA GHASEMI,[7] MOHAMMAD REZA ALAEI,[8] SETILA DALILI,[9] MAJID AMINZADEH,[10] PEGAH KATIBEH,[11] ANIS AMIRHAKIMI,[1] NEGAR YAZDANI,[1] HOMA ILKHANIPOOR,[2] ZHILA AFSHAR,[1] FATEMEH HADIPOUR,[12] ZAHRA HADIPOUR [13]

[1]*Neonatal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
[2]*Department of Pediatric Endocrinology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
[3]*Department of Biochemical Genetics, Farzanegan Lab, Shiraz, Iran.*
[4]*Epilepsy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
[5]*Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
[6]*Department of Pediatric Neurology, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[7]*Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.*
[8]*Department of Pediatric Endocrinology and Metabolism, Iran University of Medical Sciences, Institute of Endocrinology and Metabolism Research Center, Tehran, Iran.*
[9]*Pediatric Endocrinology and Metabolism, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[10]*Pediatric Diseases Research Center, Guilan University of Medical Sciences, Rasht, Iran.*
[11]*Pediatric Endocrinology and Metabolism, Pediatric Department, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
[12]*Department of Pediatric Neurology, Shiraz University of Medical Sciences, Shiraz, Iran.*
[13]*Department of Clinical Genetics, Atieh Hospital, Tehran, Iran.*
Correspondence to: *Dr. Negar Yazdani, Neonatal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. yazdani.n2017@yahoo.com*
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Objective: This study aimed to find the common inborn errors of metabolism in Iranian patients with autism spectrum disorder.

Methods: In this cross-sectional multicenter study, 105 children and adolescents with autism spectrum disorder from six centers in different cities of Iran were enrolled between August, 2019 and October, 2020. Metabolic screening, including measuring plasma levels of amino acids, acylcarnitines, creatine, and guanidinoacetate, and urinary levels of organic acids, purines, and pyrimidines was performed. Other data, including age, parental consanguinity, history of seizure, developmental mile-stones, and physical examination, were also recorded.

Results: An inborn error of metabolism was found in 13 (12.4%) patients. Five patients (4.8%) had cerebral creatine deficiency syndrome, 4 (3.8%) had arginine succinate aciduria, 2-methylbutyryl glycinuria, short-chain acyl-CoA dehydrogenase deficiency, and combined methylmalonic aciduria/malonic aciduria. There was a strong association between positive metabolic evaluation and parental consanguinity, history of seizures, microcephaly, and delayed development.

Conclusions: Our results suggest that metabolic screening should be performed in the cases of autism associated with parental consanguinity, developmental delay, and a history of seizures. The assays to be considered as a screening panel include plasma or blood amino acids, acylcarnitines, creatine and guanidinoacetate, and urinary levels of organic acids.

Keywords*: Cerebral creatine deficiency syndrome, Diagnosis, GAMT deficiency, Screening*.

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Autism spectrum disorders (ASD) are characterized by abnormalities in cognitive function and social and language communication problems. Although the etiology of ASD is unclear, genetic factos are known to be involved, and environmental factors may also play a significant role [1]. In this regard, impaired methylation and mutations of *MeCP2* have been associated with ASD. Genetic polymorphisms of cytochrome P450 enzymes have also been linked to autism, specifically CYP27B1, essential for proper vitamin D metabolism [2].

Invited Commentary: Pages 177-78.

Previous studies have demonstrated an association between inborn errors of metabolism (IEM) and ASD [3,4].

A wide range of IEMs has been found in patients with ASD, including aminoacidopathies, organic acidemias, urea cycle defects, cerebral creatine deficiencies, and abnormalities of purines and pyrimidines [5]. Early detection and timely treatment of these disorders may help improve some manifestations and prevent possible complications in patients with ASD [5]. Considering very high rates of consanguineous marriages in Iran, the association of IEM and ASD may be higher than expected. This study was conducted to document the occurrence of IEMs in patients with ASD in different centers in Iran.

METHODS

In this cross-sectional multicenter study, children and adolescents with ASD referred to either neurologists, psychiatrists, or endocrinologists in six centers in different cities of Iran were enrolled from August, 2019 to October, 2020. These centers were located in Shiraz, Isfahan, Tehran, Rasht, and Ahwaz cities. Patients with autism were referred to these specialists for different reasons such as neurologic evaluation, hormonal evaluation, or routine followups.

The protocol of the study was approved by a local ethics committee, and patients were enrolled after informed consent. We used Diagnostic and Statistical Manual of Mental Diseases, 5th edition (DSM-5) criteria to confirm the diagnosis of ASD [6]. All patients with a diagnosis of ASD were included. Patients with a history of hypoxic-ischemic encephalopathy were excluded. Information including age, history of seizures, history of other known medical conditions (such as autoimmune or inflammatory diseases) developmental assessment scales, and consanguinity of parents was collected. A detailed physical examination, including a neurologic examination, was performed.

Investigations for IEMs, including measurement of plasma amino acids, plasma acylcarnitines, urine purines and pyrimidines, plasma and urine level of guanidinoacetate and creatine were done by Liquid chromatography – mass spectrometry (LC-MS/MS) (AB-Sciex API 3000 with Agilent 1100 HPLC as front end), and measurement of urine organic acids and acyl glycines by Gas chromatography-mass spectrometry (GC-MS) (Agilent 5973 mass selective detector and 6890 GC system).

Statistical analysis: Data were analyzed using the Statistical package for the social sciences (SPSS) version 22. Continuous variables were checked for normal distribution using the Shapiro-Wilk test. The mean (SD), median (IQR), frequency, and percentages were used for descriptive analysis. The means of two independent variables were compared with the Student *t* test and the chi-square test was used for categorical variables. $P<0.05$ was considered statistically significant.

Table I Baseline Characteristics of the Study Population (*N*=105)

Variable	*Value*
Age, (y)[a]	5.8 (3.4)
Male gender	73 (69.5)
Head circumference	
Microcephaly	3 (2.9)
Macrocephaly	2 (1.9)
History of seizure	8 (7.6)
Development delay	26 (24.8)
Consanguinity	13 (12.4)

All values in no. (%) or [a]mean (SD).

RESULTS

In this study, six centers from different cities in Iran collaborated, and 105 eligible patients (69.5% boys) with ASD were enrolled (**Table I**), with a median (IQR) age of 5 (4-8) years. Thirteen patients (12.4%) were products of kinship marriages, 26 cases (24.8%) had some degree of developmental delay other than speech problems, three patients (2.9%) had microcephaly, and two (1.9%) had macrocephaly. A history of seizures was present in 7.6% (*n*=8) children.

Thirteen patients (12.4%) were diagnosed to have some form of IEM. Biochemical criteria for diagnosing these diseases, and the frequency and types of IEM found among these patients are listed in **Web Table I**. **Web Table II** shows the detailed characteristics of patients with ASD and IEM.

There was a statistically significant association between the presence of an IEM and parental consanguinity ($P<0.001$), history of seizure ($P=0.004$), and delayed development (other than speech) ($P=0.016$) (**Table II**). Among eight patients with a history of seizures, four had cerebral creatine deficiency syndrome (CCDS). There was a significant association between CCDS and history of seizure ($P=0.013$), consanguinity ($P=0.001$), and delayed development (cognitive and motor) ($P=0.04$). However,

Table II Results of Metabolic Assay in Iranian Children With Autism (*N*=105)

Variable	*Positive metabolic assay*	*P value*
Male gender, *n*=73	9 (12.3)	0.60
Microcephaly,*n*=3[a]	1 (33.3)	0.49
Seizure history, *n*=8	4 (50)	0.004
Development delay, *n*=26	7 (26.9)	0.016
Consanguinity, *n*=13	7 (53.8)	<0.001

Data in no. (%). [a]None of the two children with macrocephaly had positive metabolic assay.

WHAT THIS STUDY ADDS ?

- Cerebral creatine deficiency syndrome (CCDS) was a common type of inborn error of metabolism associated with autism among children in Iran.
- Screening is likely to be more important when autism is associated with a history of seizure, delayed development, or parental consanguinity in this population.

this association was not significant with head circumference (*P*=0.22).

DISCUSSION

Our multicenter study on 105 patients with ASD revealed a significant association between IEM and ASD in Iran. This association between IEM and ASD was found in other studies previously [7]. It may be promising to discover an IEM in a patient with ASD because, with IEM management, symptoms of autism probably ameliorate. However, this will need more extensive prospective studies from various settings [6,8,9].

Based on our results, the most prevalent type of IEM associated with ASD was CCDS. The prevalence of ASD is different in three types of CCDS including glycine amidinotransferase (AGAT) deficiency, guanidinoacetate methyltransferase (GAMT) deficiency, and *SLC6A8* deficiency or creatine transporter defect (CTD) [8]. According to previous studies,78-95% of patients with GAMT deficiency have symptoms of autism, whereas only 41% of patients with CTD have these symptoms [10,11]. It has been reported that management of GAMT deficiency can improve these patients' symptoms, such as seizures and movement disorders [3].

Phenylketonuria (PKU) and hyperphenylalaninemia are labeled as the most common types of IEM associated with autism [8,12]. However, this was not the case in our study; probably because of early detection and management through the neonatal screening program. The other metabolic disorders diagnosed by us have also been previously reported to be associated with ASD [3,12].

Although, previous authors proposed metabolic screening in only patients with ASD associated with other abnormalities [5,13,14], a recent study suggests IEM screening in all patients with ASD [12]. It is not documented whether early diagnosis and timely treatment of different metabolic disorders can improve symptoms of autism; nevertheless, some studies have reported a partial improvement in autism symptoms after starting treatment [8]. Although, CCDS has diverse signs and symptoms, including intellectual defect, neurodevelopmental delay, epilepsy, autism, and motor dysfunction, their pathophysiology is not well defined. However, the creatine-phosphocreatine system has an essential action for ATP production in the body [3] and creatine-phosphocreatine system malfunction may cause significant defects in neural signal production [15].

There were some limitations in our study. Although, the literature shows that different autism-related metabolic disorders and genetic factors are frequently associated with autism, and biotinidase deficiency and mucopolysaccharidosis are important metabolic causes, we could not evaluate for these disorders in our study due to financial constraints. Furthermore, due to the high cost of genetic testing and some limitations of accessibility of this in Iran, genetic confirmation was performed only for some patients.

Since metabolic screening in patients with ASD is not a routine practice in many countries, IEM diagnosis can be delayed, and irreversible neurologic damage can ensue. We underscore the need for further studies in different population groups regarding the yield of metabolic screening for patients with ASD, especially in communities with high rates of consanguineous marriages.

Ethics approval: Local Ethics Committee, Shiraz University of Medical Sciences; No. IR.SUMS.MED.REC. 1399.026, dated April 8, 2020.
Contributors: HM, SI, ShM: study concept and design, and preparation of the manuscript. NY: analysis and interpretation of data and the revision of the manuscript. All authors participated in data acquisition. All authors approved the final version of the manuscript and are accountable for all aspects related to the study.
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Note: Additional material related to this study is available with the online version at *www.indianpediatrics.net*

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Web Table I Diagnostic Criteria for Diagnosis of Various Inborn Errors of Metabolism Found in Children with ASD (*N*=13)

Disorder	***n (%)***	***Diagnostic criteria***
Creatine transporter defect	5 (4.7)	Severely increased urinary ratio creatine to creatine and/or pathogenic variants in the *SLC6A8* gene
Guanidinoacetate methyltransferase deficiency	4 (3.8)	The urinary ratio of guanidine acetate to creatine severely increased and the urinary ratio of creatine to creatine: decreased and/or pathogenic variants in the *GAMT* gene
Argininosuccinate lyase deficiency	1 (0.95)	Increased plasma level of arginine succinic acid and citrulline+pathogenic mutation in *ASL* gene
2 methylbutyryl glycinuria	1 (0.95)	Very high levels of methylbutyryl carnitine in plasma and 2-methylbutyryylglycine and 2-hydroxyglutaric acid in urine
Combined methylmalonic aciduria/malonic aciduria	1 (0.95)	Increased urinary levels of methylmalonic acid and malonic acid
Short-chain acyl-CoA dehydrogenase deficiency	1 (0.95)	Very high levels of butyryl carnitine and isobutyl carnitine in plasma, and ethylmalonic acid and methyl succinic acid in urine

SLC2A8:Solute carrier family 2 members; ASL: Arginosuccinate lyase.

Web Table II Characteristics of Patients with Autism and Inborn Errors of Metabolism (*N*=13)

Disorder	***Age (y)***	***Seizure***	***Development***	***consanguinity***
CTD	4, M	N_O	Normal	No
CTD	5, M	N_O	Delayed	Yes
CTD	2, M	N_O	Normal	No
CTD	3, F	N_O	Normal	No
CTD	4, M	N_O	Delayed	Yes
GAMT deficiency	6, M	Yes	Delayed	Yes
GAMT deficiency[a]	2, M	N_O	Delayed	Yes
GAMT deficiency	3, M	Yes	Delayed	Yes
GAMT deficiency	5, M	Yes	Delayed	Yes
Combined MMA/MA	4, M	N_O	Normal	No
ASA	3, M	Yes	Delayed	Yes
2MBG	9, F	N_O	Normal	No
SCAD	11, F	N_O	Normal	No

ASD; Autism spectrum disorder, IEM; Inborn error of metabolism, CTD: Creatine transporter defect; GAMT: Guanidino acetate methyltransferase; MMA/MA: Methylmalonic aciduria/malonic aciduria; ASA: Argininosuccinate aciduria; 2MBG 2 Methylbutyryl glycinuria; SCAD: Short-chain acyl-CoA dehydrogenase deficiency. All patients had normal head circumference except [a]one with microcephaly.