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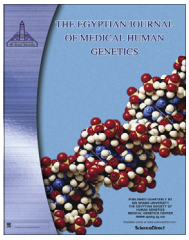

ORIGINAL ARTICLE

Study of plasma amino acid levels in children with autism: An Egyptian sample

Farida M. ElBaz [a], Mona M. Zaki [b], Azza M. Youssef [a,*], Ghada F. ElDorry [c], Dina Y. Elalfy [c]

[a] *Pediatrics Department, Ain Shams University, Cairo, Egypt*
[b] *Clinical Pathology Department, Ain Shams University, Cairo, Egypt*
[c] *Institute of Postgraduate Childhood Studies, Ain Shams University, Cairo, Egypt*

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KEYWORDS

Plasma amino acids;
Protein electrophoresis;
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Abstract *Background:* The aetiology of autism is unclear and autistic symptoms had been attributed to an abnormal functional imbalance in neurotransmitter amines such as dopamine, noradrenaline and serotonin.

Objective: To study plasma essential and non-essential amino acid levels, protein electrophoresis, serum ammonia, and urea in autistic children in comparison with controls.

Methods: Twenty autistic children were compared to twenty healthy age and sex matched normal children serving as control, where serum amino acids, urea, ammonia and protein electrophoresis were estimated.

Results: As regards essential amino acid levels, autistic children had significant lower plasma levels of leucine, isoleucine, phenylalanine, methionine and cystine than controls ($P < 0.05$),while there was no statistical difference in the level of tryptophan, valine, threonine, arginine, lysine and histidine ($P > 0.05$). In non-essential amino acid levels, phosphoserine was significantly raised in autistic children than in controls ($P < 0.05$). Autistic children had lower level of hydroxyproline, serine and tyrosine than controls ($P < 0.05$). On the other hand there was no significant difference in levels of taurin, asparagine, alanine, citrulline, GABA, glycine, glutamic acid, and ornithine ($P > 0.05$).

There was no significant difference between cases and controls as regards the levels of urea, ammonia, total proteins, albumin and globulins (alpha 1, alpha 2, beta and gamma) ($P > 0.05$).

* Corresponding author. Tel.: +20 1001409240.
E-mail addresses: faridabaz@hotmail.com (F.M. ElBaz), monazaki20@hotmail.com (M.M. Zaki), azzayos@yahoo.com (A.M. Youssef), ghadaeldorry@yahoo.com (G.F. ElDorry), dinaelalfy8@gmail.com (D.Y. Elalfy).

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Conclusions: Autistic children had lower levels of some plasma amino acids except for glycine and glutamic acids and phosphoserine were increased with normal serum levels of urea, ammonia, total proteins, albumin and globulins (alpha 1, alpha 2, beta and gamma).

1. Introduction

Autism is a complex neurodevelopment disability that is usually diagnosed before the age of three years. It is characterised by deficits in social reciprocity and in language skills that are associated with repetitive behaviours and restricted interests [1]. It affects more males than females [2].

Essential amino acids cannot be made by the body, but must be obtained in the diet. One of the problems identified with autism is a digestive system that cannot fully break down all proteins into its basic components, the amino acids and the body will only use amino acids to make systemic protein. This means that many necessary amino acids are unavailable to make systemic proteins such as metallothionine (MT) [3].

MT is a biologically essential protein that has shown to be heavily involved in the metal regulation of zinc and copper as well as the chelating of toxic metals such as cadmium, mercury and lead. MT proteins also assist in immune function, neuronal development, heart protective, brain cell protective, involved in liver cell proliferation, the absorption of nutrients in the small intestine, the breakdown of certain dietary proteins, cellular respiration, neuronal development, and energy metabolism and have antioxidant properties. Studies showed that MT could not function properly in autistic children, although it is still unclear whether this is due to genetic factors or simply low levels of MT in the body [4].

A recent study found increased plasma levels of the neuroexcitatory amino acids (as glutamic and aspartic acids) in children with autism [5]. Croonenberghs et al. 2002 found significantly increased concentrations of total serum proteins (TSP) in autistic subjects, which were attributable to increased serum concentrations of albumin and gamma globulin [6]. A study of Saudi autistic children found increased levels of ammonia and marked reduction in urea concentration [7].

2. Subjects and methods

2.1. Study population

This cross sectional case–control study was conducted on twenty autistic children 19 males (95%) and one female (5%). Their age ranged 2–7 years, (mean age 4.65 ± 1.67 years) diagnosed by DSM-IV-TR [8] and ICD-10 [9], they were graded by childhood autism rating scale (CARS) [10]. They were recruited from two clinics; the child and adolescent Psychiatric clinic, Children's Hospital, and the Institute of Postgraduate Childhood Studies, Ain Shams University, Cairo, Egypt from February 2012 to July 2013. Autistic patients were compared to twenty healthy age- and sex matched non autistic children (9 males and 11 females) serving as controls. Their mean age was 4.65 ± 1.67 years (range: 2–7 years). They were recruited from the Paediatrics' Outpatient Clinic of the same hospital. The work has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Parents of all subjects were informed of the aim and methods of the study and signed a written consent. Also approval of the ethics committee – of the university was taken.

2.2. Methods

All participants were subjected to:

- Thorough history taking with special emphasis on perinatal, developmental, vaccination, and neurological history. Thorough clinical examination with special emphasis on neurological examination.
- Evaluation of autism in autistic children by the Diagnostic and statistical manual of mental disorders 4th edition DSM-IV-TR for diagnosis of autistic disorder [8].
- Psychiatric interview for the diagnosis of autism according to the International Classification of Diseases version-10 (ICD-10) for classification of mental and behavioural disorders: This is a semi-structured instrument intended for clinician's assessment of psychiatric symptoms and syndromes in the F0–F6 categories of the ICD-10. It consists of a face sheet, screened and modules. Each module consists of a symptoms' list that may help the user to check the presenting symptoms plus considering other possible syndromes and hence the use of other modules in the checklist according to the instructions given. The user should be familiar with the ICD-10 diagnostic criteria [9].
- Application of childhood autism rating scale (CARS) for the assessment of the severity of autistic symptoms {Children with score from 15 to 30 are identified as non-autistic, from 31 to 33 are mildly autistic, from 34 to 36 are moderately autistic and from 37 to 60 are severely autistic} [10].
- Intelligence Quotient (IQ) was assessed using Wechsler intelligence scale for children both verbal and non verbal versions. IQ < 20: Profound MR, IQ 20–34: Severe MR, IQ 35–49: Moderate MR, IQ 50–69: Mild MR, IQ 70–79: Borderline IQ, IQ 80–89: Below average IQ, IQ 90–109: Average IQ, IQ 110–119: Above average IQ [11].

Laboratory investigations included:

1. Estimation of amino acids, urea and ammonia by high performance liquid chromatography (HPLC). Morning fasting blood samples were taken into 4.5-ml lithium heparin vaccutainer tubes and centrifuged at 2003*g* for 15 min to obtain platelet-rich plasma. The plasma was decanted and stored at 220 °C until analysis [12].
2. Proteins electrophoresis showing: total serum proteins, albumin, alpha, beta and gamma globulins [13].

2.3. Statistical analysis

The data were coded, entered and processed on computer using SPSS (version 15). The level $P < 0.05$ was considered the cut-off value for significance. Data are presented as median and range for continuous variables and as count and percentage for categorical variables. Groups of patients and controls were compared using Fisher's exact test for categorised variables and using Mann–Whitney U test for continuous variables. Spearman's correlation coefficient: used in Correlation between CARS, IQ and other measured parameters.

3. Results

In autistic children, Intelligence Quotient (IQ) in 10% of them was moderate mental retardation (MR), 75% had mild MR and 15% has below average IQ. As regards CARS 20% were mild autistic, 15% were moderate autistic and 65% were severe autistic and all our patients were positive for ICD10. So, there was a significant negative correlation between IQ and CARS ($P < 0.0001$).

Although in the control group 15.4% had below average IQ, 46.2% had normal IQ and 38.5% had above average IQ, they all were in non-autistic range as regards CARS and all were negative for ICD-10 Fig. 1.

As regards essential amino acid levels, autistic children had significantly reduced levels of leucine, isoleucine and phenylalanine than controls ($P < 0.05$), while there was statistical non-significant decreased level of tryptophan, valine, threonine, arginine, lysine and histidine ($P > 0.05$). In sulphur containing amino acids there was significant decreased level of methionine and cystine (Tables 1 and 2).

As regards non-essential amino acid levels, phosphoserine is significantly raised in patients than controls ($P < 0.05$). All autistic children had reduced levels of hydroxyproline, serine and tyrosine than controls ($P < 0.05$). While there was statistical non-significant decreased levels as regards taurin, asparagine, alanine, citrulline, GABA and ornithine ($P > 0.05$). Also there was statistical non-significant increased level of glycine and glutamic acids ($P > 0.05$) (Table 3).

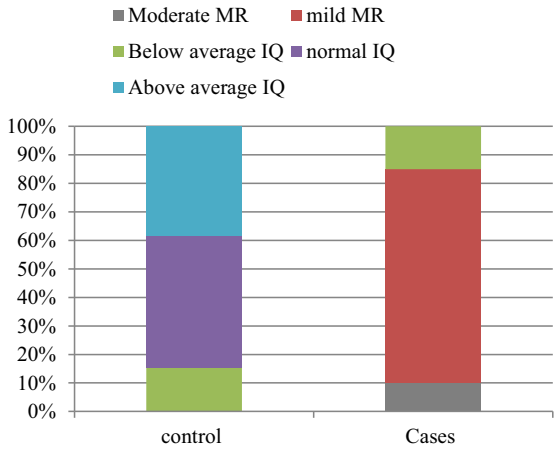

Figure 1 Statistical comparison between cases and control as regards IQ distribution.

There was no statistical significant difference between cases and controls as regards the levels of urea, ammonia, total proteins, albumin, alpha 1, alpha 2, beta, gamma globulins and albumin/globulin ratio (using protein electrophoresis) ($P > 0.05$) (Table 4).

In our study the higher the CARS score the lower level of some essential amino acids was explained by a significant negative correlation between CARS and leucine ($p = 0.002$), isoleucine ($p = 0.02$), phenylalanine ($p = 0.003$), methionine ($p = 0.001$), cysteine ($p = 0.003$), serine ($p = 0.002$) and tyrosine ($p = 0.01$) (Table 5).

4. Discussion

Autism spectrum disorders (ASD) are common complex neurodevelopmental conditions. Diagnostic criteria for these conditions have traditionally relied solely on behavioural criteria without consideration for potential biomedical underpinnings [1].

In the present study all autistic children had lower IQ than in controls, 75% of them were mentally retarded with their IQ range of 50–70. This is in agreement with Bolte et al. [14] who found autistic children to have lower IQ than controls using WISC. Also Nelson [15] found that the majority of autistic children (approximately 67–88%) are definitely mentally retarded with their IQ usually below 70.

In the present study all autistic children had high scores of CARS (more than 30).The same was also reported by Pilosky et al. [16].

In the current study there was a significant negative correlation between IQ and CARS (correlation coefficient 0.74) $p < 0.0001$. Mclnne et al, [17] also reported that the severity of mental retardation was seen in most of their severe cases of autism.

As regards essential amino acids in our study there was a significantly lower level of leucine, isoleucine and phenylalanine in autistic children than controls. The decreased phenylalanine level may lead to lower concentrations of catecholamines such as dopamine, adrenaline, and noradrenaline, with subsequent effects on behaviour. This is in agreement with another study which demonstrated deficiencies of leucine, isoleucine and phenylalanine in autistic children [18]. On the other hand Aldred et al. [19] and Alam et al. [20] demonstrated that autistic children, their siblings and parents had raised level of phenylalanine, these results show that autistic children come from a family background of dysregulated amino acid metabolism.

In our study serum tryptophan level was non-significantly decreased in cases than controls. This comes in agreement with the study done by Moreno-Fuenmayor et al. [5].

On the other hand Hoshino et al. [21] demonstrated significantly higher levels of plasma free tryptophan in autistic children than in normal control subjects. These findings suggest the presence of some defects in the metabolism of tryptophan in the brain of autistic children and needs further studies.

As regards sulphur containing amino acids, in the present study there is a significantly lower levels of methionine and cysteine levels in all autistic children than controls. This is in agreement with Johns et al. [22] who found that autistic children had significantly lower baseline plasma concentration of methionine and cysteine than in control children. Also Geier and Geier [23] confirmed our results. This may be due to the

Table 1 Statistical comparison between cases and controls as regards essential amino acids.

Essential amino acids	Patients (20)			Control (20)			*Z*	*P*	Sig.
	Median	IQR		Median	IQR				
Valine	67.49	50.59	95.20	75.56	42.52	134.62	1.00	0.31	NS
Leucine	35.66	15.21	48.14	64.08	49.96	85.88	3.24	0.001	S
Isoleucine	21.35	6.61	24.69	29.49	18.05	33.61	1.95	0.05	S
Threonine	30.36	8.26	55.34	34.39	22.19	60.55	0.66	0.51	NS
Phenylalanine	22.21	11.46	35.41	44.51	34.28	53.83	3.12	0.002	S
Tryptophan	1.19	0.33	6.43	1.54	0.48	5.77	0.08	0.94	NS
Arginine	6.95	4.76	57.25	17.51	2.67	41.70	0.07	0.94	NS
Lysine	49.23	14.38	1096.34	67.01	43.18	95.74	0.12	0.91	NS
Histidine	5.57	2.96	8.36	44.13	13.40	60.53	1.74	0.08	NS

Amino acids are measured by micro mol/L. IQR: inter quartile range, NS = non significant, S = significant.

Table 2 Statistical comparison between cases and controls as regards sulphur containing amino acids:

Sulphur cont. amino acids	Patients (20)			Control (20)			*Z*	*P*	Sig.
	Median	IQR		Median	IQR				
Methionine	5.95	2.56	11.45	17.38	10.19	25.44	2.91	0.004	S
Cystine	3.44	0.98	9.07	17.79	10.23	19.73	2.77	0.006	S

Amino acids are measured by micro mol/L, S = significant.

Table 3 Statistical comparison between cases and controls as regards non-essential amino acids.

Non essential amino acids	Patients (20)			Control (20)			*Z*	*P*	Sig.
	Median	IQR		Median	IQR				
P. serine	7.50	4.44	14.76	3.20	2.90	8.31	2.17	0.03	S
Taurin	60.75	46.46	118.55	91.90	62.71	171.18	1.62	0.10	NS
H. proline	10.06	0.46	70.97	1568.67	36.69	2637.22	2.44	0.01	S
Serine	36.19	5.76	51.62	69.10	38.53	133.07	2.45	0.01	S
Asparagine	84.86	30.28	1168.88	107.66	48.56	398.28	0.00	1.00	NS
Alanine	143.17	98.35	184.95	224.40	49.74	671.76	0.81	0.42	NS
Proline	42.42	13.22	138.84	76.87	50.88	489.83	1.52	0.13	NS
Glycine	101.71	81.69	123.92	91.72	56.86	122.84	0.49	0.62	NS
Citrulline	25.41	10.70	100.03	70.09	5.39	125.37	0.00	1.00	NS
Tyrosine	22.12	7.53	38.11	33.72	26.89	60.70	2.20	0.03	S
GABA	7.96	3.05	29.44	9.62	4.97	21.53	0.90	0.37	NS
Ornithine	93.55	63.87	215.42	100.91	70.09	164.93	0.08	0.94	NS
Glutamic acid	62.65	21.87	141.44	56.13	21.68	105.85	0.43	0.67	NS

The amino acids are measured by micro mol/L, H. Proline = hydroxyl proline, NS = non significant, S = significant.

Table 4 Statistical comparison between cases and controls as regards urea, ammonia and protein electrophoresis.

	Patients (20)			Control (20)			*Z*	*P*	Sig.
	Median	IQR		Median	IQR				
Urea	7.58	5.33	9.91	6.85	4.57	9.13	0.66	0.51	NS
Ammonia	2.40	0.57	7.65	1.98	1.30	2.65	0.57	0.57	NS
Total pr	7.15	6.53	7.98	7.40	6.95	7.95	0.68	0.49	NS
Albumin	3.90	3.53	4.58	4.00	3.80	4.35	0.61	0.54	NS
Alpha 1	0.20	0.20	0.20	0.30	0.15	0.30	1.24	0.22	NS
Alpha 2	1.10	0.93	1.30	1.20	1.10	1.30	0.62	0.54	NS
Beta	1.00	0.90	1.18	1.00	1.00	1.15	0.66	0.51	NS
Gamma	0.90	0.83	1.08	1.00	0.80	1.15	0.47	0.64	NS
A/G ratio	1.23	1.16	1.31	1.17	1.07	1.33	0.55	0.58	NS

Amino acids, urea and ammonia are measured by micro mol/L.
Proteins in protein electrophoresis are measured in g/dL.

Table 5 Correlation between CARS and amino acid levels among cases.

	CARS		
	Correlation coefficient	*P*	
Valine	−0.16	0.38	NS
Leucine	−0.54*	0.002	S
Isoleucine	−0.43*	0.02	S
Threonine	−0.37	0.07	NS
Phenylalanine	−0.51*	0.003	NS
Tryptophan	0.17	0.47	NS
Arginine	−0.17	0.45	NS
Lysine	−0.11	0.61	NS
Histidine	−0.35	0.20	NS
Methionine	−0.58*	0.001	S
Cysteine	−0.62*	0.003	S
P. serine	0.25	0.17	NS
Taurine	−0.23	0.19	NS
Hproline	−0.36	0.07	NS
Serine	−0.53*	0.002	S
Asparagine	0.08	0.72	NS
Alanine	−0.20	0.34	NS
Proline	−0.38	0.07	NS
Glycine	0.04	0.85	NS
Citrulline	−0.24	0.54	NS
Tyrosine	−0.45*	0.01	S
GABA	−0.03	0.86	NS
Ornithine	−0.03	0.89	NS
Glutamic acid	−0.18	0.44	NS

NS = non significant, S = significant.
* $P < 0.05$ significant.

malfunction of the digestive system in autistic children including leaky gut and food allergies due to low levels of sulphur [23].

In the present study the non-essential amino acids as phosphserine, glutamic acid and glycine were significantly increased in cases versus controls as well as decreased levels of the rest of non-essential amino acids especially hydroxyproline, serine and tyrosine in patients than controls. The same was also reported by Zavala et al. [24] and Moreno-Fuenmayor et al. [5] as they found that there was a significant difference between patients and controls regarding the levels of glutamic acid which was significantly higher in patients than controls.

In the present study there was a significant negative correlation between CARS and some of the decreased amino acids such as leucine ($p = 0.002$), isoleucine ($p = 0.02$), phenylalanine ($p = 0.003$), methionine ($p = 0.001$), cysteine ($p = 0.003$), serine ($p = 0.002$) and tyrosine ($p = 0.01$) so the higher the CARS score the lower level of these amino acids. This was Confirmed by Adams et al. [25] who found in most of autistic cases the lower amounts of essential amino acids were correlated with more severe autistic features.

In our study there was no statistical significant difference in protein electrophoresis results between cases and controls as regards the levels of total proteins ($p = 0.49$), albumin ($p = 0.54$), alpha 1 globulin ($p = 0.22$), alpha 2 globulin ($p = 0.54$), beta globulin ($p = 0.51$), gamma globulin ($p = 0.64$) and A/G ratio ($p = 0.58$). Both cases and controls had normal levels. On the other hand Croonenberghs et al. [6] found significantly increased concentrations of TSP in autistic subjects, which were attributable to increased serum concentrations of albumin and gamma globulin. Serum IgG, IgG2 and IgG4 were also significantly raised. Also in their study, there were significant and positive correlations between social problems and TSP and serum gamma globulin and between withdrawal symptoms and TSP and serum albumin and IgG. The controversy between our and their results may be due to small sample size or limitation of the study. This issue needs more studies as no more researches concerning this issue were found.

Abu Shmais et al. [7] in a study of Saudi autistic children found increased levels of ammonia and marked reduction in urea concentration in contrary to our results where ammonia and urea levels were normal. This controversy may be due to limitation of our study or small sample size and also need more researches to discuss it.

In conclusion, autistic children may have dysregulated amino acids metabolism as all amino acids except for glutamic acid, phosphoserine and glycine are decreased in patients than in control; the raised glutamic acid may suggest involvement of an altered glutamate transporter and is consistent with a biochemical basis for autistic disorders. Also, the lower amounts of essential amino acids are correlated with more severe autism.

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