Autism Spectrum Disorders: The Association with Inherited Metabolic Disorders and Some Trace Elements. A Retrospective Study

Article *in* CNS & neurological disorders drug targets · April 2019

DOI: 10.2174/1871527318666190430162724

3 authors, including:

Wafaa Moustafa M. Abo El Fotoh
Menoufia University
32 PUBLICATIONS **24** CITATIONS

SEE PROFILE

Nahla M Said Abd El Hady
Minoufiya University
7 PUBLICATIONS **49** CITATIONS

SEE PROFILE

Autism Spectrum Disorders: The Association with Inherited Metabolic Disorders and Some Trace Elements. A Retrospective Study

Wafaa Moustafa M. Abo El Fotoh, Sameh Abdallah Abd el naby, Nahla M Said Abd El Hady

Abstract

Background: Autism Spectrum Disorders (ASD) as a considerable health obstacle in kids is characterized by compromised social collaboration, and stereotyped behavior. Autism is triggered by an interactive impact of environmental and genetic influences. Presumablysome inborn errors of metabolism are implicated in a sector of developmental disabilities. Also, several trace elements may have an important role in human behavior, and neurological development. This study was designed to verify the frequency of inherited metabolic disorders and /or trace element abnormalities in children with ASD.

Methods: In a retrospective analytical study, 320 children diagnosed with ASD according to the DSM-V criteria and Childhood Autism Rating Scale criteria were enrolled in this study. Serum ammonia, blood lactate, and arterial blood gases, plasma amino acid profile by tandem mass spectrophotometry, and a urinary organic acid assay were performed in all the patients. Likewise, the estimation of a number of trace elements in the form of serum lead, mercury, copper, and plasma zinc was done in all the patients.

Results: A total of 320 children with ASD, inherited metabolic disorders were identified in eight (2.5%) patients as follows**:** Seven (2.19) patients with phenylketonuria, and one (0.31%) patient with glutaric aciduria type 1. As regards the trace element deficiency, sixteen (5%) patients presented low plasma zinc level, five (1.56%) children presented a high serum copper level, two (0.62%) children presented a high serum lead level and only one (0.31%) autistic child presented high serum mercury level. Electroencephalogram (EEG) abnormalities were reported in 13.12% and Magnetic Resonant Imaging (MRI) abnormalities in 8.43% of cases.

Conclusion: Screening for metabolic diseases and trace elements is required in all children diagnosed with ASD irrespective of any apparent clinical attributes of metabolic complaints and trace elements discrepancies.

Keywords: ASD, Metabolism, Phenylketonuria, Screening, Trace elements, Zinc.

1. Introduction

Autism is an extremely inconsistent neurodevelopmental disorder,which typically develops during infancy or early childhood, with a subsequently steady pattern of progression. There is generally no remission, and the presentation requires difficulties in social contact and communication with repetitive and restricted patterns of behaviour [1-2]. The reported prevalence of autism has shown a significant increase in recent years. This is due, in part or entirety, to factors such as advances in diagnostic methods, agreed-upon diagnostic guidelines, increasing access to facilities improved clinician and public awareness, and early age identification [3]. Statistically the prevalence of autism has increased to 1-2/1,000 and about 6/1,000 for ASD [4].

Several processes are being elucidated in the pathophysiology of autism. There is growing evidence regarding a variety of developmental impacts that can affect the brain function [5]. A growing range of genetic factors and multifactorial causes can be involved in ASD development [6]. Liu et al. strongly suggest that ASD may involve not only organ-specific abnormality, but also systemic abnormalitiesand specific organ dysfunctionsuch as immune impairments, inflammation, reduced detoxification, environmental toxic acquaintances, redox regulation/oxidative stress, mitochondrial dysfunctionand gut disorders [7]

Inherited Metabolic Disorders (IMD) have been reported in approximately 2-5% of patients with ASD [8]. Phenylalanine is an essential amino acid used in a variety of cellular proteins. Its metabolite, tyrosine, is a dopamine and a melatonin precursor in the human brain [9]. Elevated phenylalanine levels have a neurotoxic effect, leading to structural brain injury, cognitive incompetence, and behavioural disruptions, as seen in untreated individuals with Phenylketonuria

(PKU) [10]. Metabolic syndrome has been proposed as a risk factor for cerebrovascular disorders and has been linked with cognitive dysfunction in many functional brain areas [11].

On the other hand, trace elements play a significant role in cell function. Most trace elements behave as cofactors or mediators for enzymes involved in key biochemical processes. These elements are also important for the synthesis and stability of many cell components such as enzymes and tissue proteins. The excess accumulation or deficiency of such elements may foster an alternate metabolic pathway which can result in many diseases and neurodevelopment disorders [12-13]. This study was proposed to examine the occurrence of inherited metabolic and/or trace element disorders in a representative sample of autistic children.

2. Material and Methods

2.1. Study design / Participants

The records of 320 children diagnosed with autistic disorder (228 males and 92 females) followed up in the Pediatric Neurology Clinic, Menoufia University Hospitals between January 2015 and February 2017, were retrospectively gathered to estimate the prevalence of underlying metabolic and trace element disorders in this Egyptian cohort. All children who had a secondary cause for ASD, such as genetic syndromes, a formerly diagnosed IMD, Central nervous system (CNS) infection or tumor, and a history of perinatal injury were excluded from the study. The diagnosis was confirmed by using the Diagnostic and Statistical Manual V (DSM-V) criteria and Childhood Autism Rating Scale (CARS) for the diagnosis and screening of childhood ASD. The study was approved by the Ethical Committee of Menoufia University. A written consent in harmony with the Ethical Committee approvals was retained by all of the caregivers of the children enrolled in the study.

2.2. Data collection/work-up

Data collection included detailed family records (e.g. Consanguinity, similarly affected personnels, neurodevelopmental disorders and psychiatric disorders). Medical history, physical and neurological examinations have been also documented. Laboratory work-up comprised of a complete blood count, serum electrolytes, random blood glucose, liver function tests, kidney function tests, arterial blood gases, measurement of serum ammonia, and blood lactate. Metabolic investigations included quantification of plasma amino acid by tandem mass spectrometry and urinary organic acid profile. Estimation of three trace elements in the serum (lead, mercury, and copper) as well as plasma zinc measurement. Neuroimaging studies in the form of Magnetic Resonant Imaging (MRI) and Electroencephalography (EEG) were carried out based on the clinical facts.

2.3. Statistical analysis

The data were analyzed statistically by the statistical package for social sciences 22 (SPSS inc., Chicago, IL, USA). For quantitative variables, the data were expressed as a mean± standard deviation.

3. Results

A total of 320 patients with autistic disorder, 228 patients were males (71.25%), and 92 patients were females (28.75%) with males outnumbered females where the ratio was 2.47/1, respectively. The mean age of the onset of diagnosis was 6.77±2.45 years and ranged from 3 to 16 years. 126 patients (39.37%) showed a positive consanguinity. Epilepsy was identified in 53 children (16.56%) [see Table 1].

Inherited metabolic disorders were identified in eight patients as follows: Seven patients were diagnosed with phenylketonuria (2.19%), one patient was diagnosed with glutaric aciduria type 1 (0.31%). Thus, the prevalence of IMDs among our kids with ASD was 2.5% [fig.1]. The detailed description of all cases diagnosed with IMDs is illustrated in Table 2.

As regards the trace elements, sixteen patients (5%) displayed low plasma zinc levels, three of them demonstrated frequent hospital admission due to gastroenteritis. Five patients (1.56%) had higher copper levels, but their ceruloplasmin and 24 urine copper were normal. Decreased socialization was the main presentation in four of autistic children with elevated serum copper

levels. Two children (0.62%) complaining speech delay had higher lead levels above the normal values and only one autistic female child with decreased socialization revealed a high mercury level. Their clinical description is detailed in Table 3.

4. Discussion

Many IMDs have been seen with autism spectrum disorders at a rate more than the general population [14]. Phenylalanine metabolic disorders have been identified in kids with autism spectrum disorders and epilepsy [15]. Seven patients (2.19%) from 4 to 13 years of age were diagnosed with PKU in the current study. In Egypt, the nationwide screening for PKU in newborns began in 2016; however, our enrolled patients were born before the initiation of this nationwide screening.

Phenylketonuria is an autosomal recessive disorder caused due to a mutation in the phenylalanine hydroxylase gene, located on chromosome 12q23.2. A newborn screening identifies babies with PKU, and facilitates the introduction of certain dietary choices. Infants born with PKU who strictly follow a diet can be assumed to live a normal life. On the contrary, children who go away unmanaged or who do not sufficiently adhere to the diet may display retarded growth, fair skin, small head size, convulsions, hypertonia, hyperactive behavior, autistic manifestations, developmental delay, and severe cognition difficulties [15]. Baieli and others observed that no neonates diagnosed as classic PKU met the diagnostic criteria for an autistic disorder; however, 6% of the children with a late diagnosis of classic PKU were identified as having an autistic disorder. [16].

The cognitive impairment that can be observed in patients with phenylketonuria" is assumed to be a consequence of dopamine depletion in the prefrontal cortex. The amino acid phenylalanine competes with tyrosine to overcome the blood-brain barrier, but the transporting molecules display more affinity for phenylalanine than for tyrosine, which is essential for the synthesis of dopamine [17]. Furthermore, Glushakow and others stated that the elevated phenylalanine levels substantially reduce the action of glutamate receptors in excitatory synapses by three mechanisms: reduction of glutamate discharge at the presynaptic neurons; opposition against glycine binding sites of the N-methyl-D-aspartate (NMDA) receptors; presence of antagonists at the glutamate binding site of non-NMDA receptors [18]. Too much binding of NMDA receptors to glutamate permits calcium entry into postsynaptic neurons and results in brain cell death. Contrariwise, the binding of excess glutamate to non-NMDA receptors bring about sodium entry into postsynaptic neuron and the resultant cytotoxic edema. Furthermore, the glial neuronal cells bearing these receptors lose the ability to protect brain neurons from glutamate excitotoxicity and brain damage [19]. Likewise, phenylalanine inhibits Na-K ATPase action in synaptosomes resulting in movement of the water from outside to inside the brain cells and lead to longstanding intracellular edema of brain tissues [20].

In our study one autistic male child diagnosed with Glutaric aciduria type I. To the best of our expertise, the association between Glutaric aciduria type 1, and autism disorder has been described in only one study carried out on 778 autistic children in Turkey, in which five years old male child with normal features except for large head size was diagnosed as Glutaric aciduria [21]. This of course warrants the screening for organic acidurias in autistic children. Previous reports have been mentioned the association between a number of organic acidemias and neuropsychatric symptoms, including Propionic and Isovaleric acidemias. Grünert et al. retrieved that 70% of the children with Propionic academia presented impairment in their psychomotor, cognitive and speech development [22]. The most common MRI abnormality was observed in the basal ganglia [23]. Also, 44 % of patients with Isovaleric academia showed mild motor dysfunction while 19% showed cognitive and neuropsychatric impairments [24].

Trace elements take an essential role in the human health and wellbeing in normal concentrations, but at abnormal levels, these elements are persumed to be harmful and disrupt the normal physiological mechanisms, resulting in various diseases [25]. Rossignol et al. reported that prenatal or postnatal environmental exposure to pro-oxidant agents such as mercury, lead,

viruses, air pollutants, and pesticides has been concerned with ASD, as these influences were combined with lower blood reduced Glutathione (GSH) concentrations, enhanced oxidative stress, reduced cellular signaling, impaired immune function and mitochondrial dysfunction [26-27].

Though, the pathophysiological associations between zinc, copper and autism disorder, particularly on a molecular background are not well appreciated. Zinc an essential trace element has a crucial role in gastrointestinal tract (GIT) and brain developments, as well as for normal immune function. Zinc deficiency has been associated with numerous neuropsychiatric disorders such as depression and autism disorder [28-29]. Sixteen patients have low zinc levels in this study, three of them showed frequent hospital admission due to gastroenteritis. Also, EEG changes were observed in four patients. Faber et al. stated that the frequency of zinc deficiency is increased in autistic children. Alsoo, Lakshmi priya and Geetha noticed a significant difference in hair and nail zinc levels of autistic children compared to the healthy ones [30-31].

The interpretation for frequent gut troubles in the autism disorder stays unclear. GIT problems might be caused by increased intestinal permeability or the leaky gut phenomenon, which has been described in children with autism spectrum disorders [30]. The current research indicates a possible linkage between the dysfunctions associated with zinc deficiency and GIT troubles as autistic children often complainof gut disorders such as gastroesophageal reflux, diarrhea and loose stools, constipation, bloating, distension and abdominal colic [31].

It has been reported that autistic children with zinc deficiency occasionally encounter EEG abnormalities. The treatment of schizophrenic patients with zinc was resulted in a decrease in EEG amplitude towards the normal activity, that was consistent with a decline in their cortical excitability [32]. Zinc deficiency can influence the vulnerability to seizures. In rats, the chelation of zinc has increased the susceptibility to kainic acid- provoked seizure activity and reversibly disrupts the hippocampal- spatial- functioning memory [33-34]. Also, lower concentrations of zinc in the hair and plasma were associated with seizures in epileptic patients [35-36]. A negative correlation has been reported between plasma zinc concentration and severity of hyperactivity and fine motor skills [36].

Copper is a metal ion which is needed for critical body functions. Depression, bad temper, irritability, anxiety, learning and behavioral disarrays are the possible neurotoxic consequences of high copper concentration [37]. Five patients in our study showed high copper levels, whereas their ceruloplasmin and 24 urinary copper levels were normal. Russo and Robert found a significant elevation of copper in the autistic children. Also, Elsheshtawy et al. noticed higher levels of copper in the autistic children than in control group [38-41]. Moreover, Russo et al. found that autistic patients had higher plasma levels of copper (108.9 μg/dL) than controls (86.5 μg/dL;$P = 0.003$). These elevated levels were associated with deficits in expressive and receptive language, focus attention, hyperactivity and fine and gross motor skills [38]. Geier et al. reported that 85% or more of autistic persons have an elevated copper level thatalso interferes with Gamma Aminobuyeric Acid (GABA) receptor function. The excess copper has a potent influence on the brain and may be correlated with schizophrenia, autism disorder, infant hyperactivity, and depressive symptoms. Geier et al. assumed that the adverse effect of this toxic metal is more apparent in children diagnosed with moderate to severe autism than in those with a mild disorder [42].

Two autistic boys had elevated lead levels in this study. Jiang et al. noticed that hair lead concentrations in autistic preschool students were considerably elevated. [43]. Also, the lead concentrations in children residing close to gas stations were somewhat greater than in children living away from such stations [44].

Mohamed et al. observed that the mercury level in Egyptian autistic children was significantly elevated over the controls [45]. Maternal fish intake during pregnancy was one of the associated risk factors. Fang et al. reported that the mercury concentration in hair was definitely correlated with the average amount of fish utilized weekly [46]. A significant relationship between the

severity of the autism disorder and the overall mercury exposure during the prenatal and early postnatal periods based on thimerosal-incorporating immunoglobulins has also been reported [47]. Only one autistic child had elevated serum mercury in our study that may have been due to the relatively small sample size or to different environmental influences. Also, hair mercury measurements are needed for a better assessment of mercury status in autistic children.

Regarding the relationship between autism and neuroimaging changes, EEG and MRI abnormalities were detected in 13.12% and 8.43% of our cases, respectively. There is an approximate 10% increase in brain volume in the early childhood period with a peak at 2-4 years of age, followed by a plateau phase [48]. A thicker brain cortex, an increased gyrification index in the left frontal lobe, and decreased cortical folding were noticed in boys with autism compared to healthy children, which was age related [48-49]. On the other hand, Kosinovsky et al. noted no parenchymal abnormalities in MRI or CT studies of 70 children with ASD, while there were significant abnormalities in children with ASD due to a specific syndrome diagnosis; thus, these abnormalities may have been due to the underlying disorder rather than to ASD [51]. For example,the Phosphatase and tension (PTEN) gene mutation plays an immense role in the physical characteristics that occur in ASD, such as macrocephaly, and in mice it has been linked with neurological modifications such as increased soma mass, axonal growth, ectopic and hypertrophic axonal projections, and altered synapses with exaggerated presynaptic varicosities [52.53].

Earlier studies revealed EEG abnormalities in 60.7% of autistic children; the generalized spike and slow wave discharges were the most frequently observed EEG activities followed by focal temporal region discharges [54]. Reinhold et al. found that the abnormal EEG findings in ASD included epileptiform abnormalities in 65% of children, and slowing in only 15% of patients [55]. Also, Gubbay et al. found that 30% of autistic children had seizures and abnormal EEG findings [56]. It is assumed that epilepsy in particular correlated well with the severity of behavioural troubles and defective cognitive performance in autistic children with only 8% of patients with ASD identified with intellectual regression in the abscence of epilepsy [57-58].

The limitations of the study: this was a retrospective study, lack of control subjects, and a comparatively small sample size. Furthermore autistic children needed to be evaluated for other environmental risk factors and trace elements.

In summary, screening for metabolic disorders, estimation of trace elements and micronutrient levels is helpful. Eradication of exposure to toxic trace elements, perinatal counseling about maternal exposure to risks of autism during pregnancy. Other studies as intestinal permeability studies, stool analysis, urinary peptides, hair analysis for trace element levels, functional neuroimaging, genetic diagnostic testing are recommended.

5. **Conclusion**: Screening for metabolic diseases and trace elements is indicated in the etiologic work-up of children with autistic disorders. Autistic children may exhibit some associated disorders such as seizures, gastrointestinal issues, and speech problems therefore, they need rapid and good intervention.

Conflicts of interest: The authors declare no financial or other conflicts of interest related to this work.

Figure legend: Fig.(1): The frequency of inherited metabolic disorders and trace elements disorders in the autistic children

References

1. Geschwind DH. Autism: many genes, common pathways? Cell 2008;135(3):391-5.
2. Landa RJ. Diagnosis of autism spectrum disorders in the first 3 years of life. Nat Clin Pract Neurol 2008;4 (3): 138–47.
3. Fombonne E. Epidemiology of pervasive developmental disorders. Pediatr Res 2009; 65 (6): 591–8.

4. Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE, Mandell DS, Miller LA, Pinto-Martin J, Reaven J, Reynolds AM, Rice CE, Schendel D, Windham GC. The epidemiology of autism spectrum disorders. Annu Rev Public Health 2007; 28: 235–58.
5. Müller RA. The study of autism as a distributed disorder. Ment Retard Dev Disabil Res Rev 2007;13 (1): 85–95.
6. Bertoglio K, Hendren RL. New developments in autism. Psychiatr Clin North Am 2009;32(1):1-14.
7. Liu H, Talalay P, Fahey JW. Biomarker-Guided Strategy for Treatment of Autism Spectrum Disorder (ASD). CNS Neurol Disord Drug Targets. 2016;15(5):602-13.
8. Ghaziuddin M, Al-Owain M. Autism spectrum disorders and inborn errors of metabolism: an update. Pediatr Neurol 2013;49 (4):232-6.
9. Blau N, Hennermann JB, Langenbeck U, Lichter-Konecki U. Diagnosis, classification, and genetics of phenylketonuria and te´trahydrobiopte´rine (BH4) deficiencies. Mol Genet Metab 2011;104:S2-S9.
10. Moyle JJ, Fox AM, Arthur M, Bynevelt M, Burnett JR. Meta-analysis of neuropsychological symptoms of adolescents and adults with PKU. Neuropsychol Rev 2007;17:91-101.
11. Martinelli I, Tomassoni D, Moruzzi M, Traini E, Amenta F, Tayebati SK. Obesity and Metabolic Syndrome Affect the Cholinergic Transmission a nd Cognitive Functions. CNS Neurol Disord Drug Targets. 2017;16(6):664-676.
12. Wada O. What are trace elements? Their deficiency and excess states. J Japan Med Assoc 2004;47:351-8.
13. Yui K. Editorial: New Therapeutic Targets for Autism Spectrum Disorders. CNS Neurol Disord Drug Targets. 2016;15(5):529-32. Review. PubMed PMID: 27174672
14. Manzi B, Loizzo AL, Giana G, Curatolo P. Autism and metabolic diseases. J Child Neurol 2008;23(3):307-14.
15. Williams RA, Mamotte CD, Burnett JR. Phenylketonuria: an inborn error of phenylalanine metabolism. Clin Biochem Rev 2008;29(1):31-41.
16. Baieli S, Pavone L, Meli C, Fiumara A, Coleman M. Autism and phenylketonuria. J Autism Dev Disord 2003;33(2):201-4.
17. Diamond A. Consequences of variations in genes that affect dopamine in prefrontal cortex. Cereb Cortex 2007; 17(1): i161-i170.
18. Glushakow AV, Dennis DM, Sumners C, Seubert CN, Martynyuk AE. L-phenylalanine selectively depresses currents at glutamatergic excitatory synapses. J Neurosci Res 2003;72: 116-124.
19. Moritani T, Smoker WR, Sato Y, Numaguchi Y, Westesson PL. Diffusion-weighted imaging of acute excitotoxic brain injury. AJNR Am J Neuroradiol 2005; 26: 216-228.
20. Kono K, Okano Y, Nakayama K, Hase Y, Minamikawa S, Ozawa N, Yokote H, Inoue Y. Diffusion-weighted MR imaging in patients with phenylketonuria: relationship between serum phenylalanine levels and ADC values in cerebral white matter. Radiology 2005; 236: 630-6.
21. Kiykim E, Zeybek CA, Zubarioglu T, Cansever S, Yalcinkaya C, Soyucen E, Aydin A. Inherited metabolic Disorders in Turkish Patients With Autism Spectrum Disorders. Autism Res 2016; 9: 217–23.
22. Grünert SC, Müllerleile S, De Silva L, Barth M, Walter M, Walter K, Meissner T, Lindner M, Ensenauer R, Santer R, Bodamer OA, Baumgartner MR, Brunner-Krainz M, Karall D, Haase C, Knerr I, Marquardt T, Hennermann JB, Steinfeld R, Beblo S, Koch HG, Konstantopoulou V, Scholl-Bürgi S, van Teeffelen-Heithoff A, Suormala T, Sperl W, Kraus JP, Superti-Furga A, Schwab KO, Sass JO. Propionic acidemia: clinical course and outcome in 55 pediatric and adolescent patients. Orphanet J Rare Dis 2013; 8: 6.

23. Grünert SC, Müllerleile S, De Silva L, Barth M, Walter M, Walter K, Meissner T, Lindner M, Ensenauer R, Santer R, Bodamer OA, Baumgartner MR, Brunner-Krainz M, Karall D, Haase C, Knerr I, Marquardt T, Hennermann JB, Steinfeld R, Beblo S, Koch HG, Konstantopoulou V, Scholl-Bürgi S, Van Teeffelen-Heithoff A, Suormala T, Sperl W, Kraus JP, Superti-Furga A, Schwab KO, Sass JO. Propionic acidemia: neonatal versus selective metabolic screening. J Inherit Metab Dis 2012;35:41–49.
24. Grünert SC, Wendel U, Lindner M, Leichsenring M, Schwab KO, Vockley J, Lehnert W, Ensenauer R..Clinical and neurocognitive outcome in symptomatic isovaleric academia. Orphanet J Rare Dis 2012;7:9.
25. Nath R. Health and Disease: Role of Micronutrients and Trace Elements. APH Publishing Corporation, New Delhi. 2000; pp: 650.
26. Rossignol DA, Genuis SJ, Frye RE. Environmental toxicants and autism spectrum disorders: a systematic review. Transl Psychiatry 2014; 4: e360.
27. Cikankova T, Sigitova E, Zverova M, Fisar Z, Raboch J, Hroudova J. Mitochondrial Dysfunctions in Bipolar Disorder: Effect of the Disease and Pharmacotherapy. CNS Neurol Disord Drug Targets. 2017;16(2):176-186.
28. Maret W. Zinc and human disease. Met Ions Life Sci. 2013;13: 389–414.
29. Iulita MF, Caraci F, Cuello AC. A Link Between Nerve Growth Factor Metabolic Deregulation and Amyloid-?-Driven Inflammation in Down Syndrome. CNS Neurol Disord Drug Targets. 2016;15(4):434-47. Review. PubMed PMID: 26996175
30. Faber S, Zinn GM, Kern JC 2nd, Kingston HM. The plasma Zinc/ serumCu2+ ratio as a biomarker in children with autism spectrum disorders. Biomarkers 2009; 14: 171-80.
31. Lakshmi Priya, Geetha A. Levels of trace elements (cu2+, Zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol Trace Elem Res 2011;142(2):148-58..
32. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, Carteni M, De Rosa M, Francavilla R, Riegler G, Militerni R, Bravaccio C. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 2010; 51:418–424.
33. Vela G, Stark P, Socha M, Sauer AK, Hagmeyer S, Grabrucker AM. Zinc in gut – brain interaction in Autism and neurological disorders. Neural Plast. 2015;2015:972791.
34. Goldstein L, Pfeiffer CC. Effects of single oral doses of trace elements on the quantitated EEG of normal subjects and chronic schizophrenics. Psychopharmacol Bull 1978;14:55-7.
35. Mitchell CL, Barnes MI, Grimes LM. Diethyldithiocarbamate and dithizone augment the toxicity of kainic acid. Brain Res 1990;50: 327-30.
36. Frederickson RE, Frederickson CJ, Danscher G. In situ binding of bouton zinc reversibly disrupts performance on a spatial memory task. Behav Brain Res 1990;38:25-33.
37. Tang SM. Hair zinc and copper concentration in patients with epilepsy. Zhonghua Shen Jing Jing Shen Ke Za Zhi. 1991;24(2):94-7, 124-5.
38. Russo AJ, Bazin AP, Bigega R, Carlson RS 3rd, Cole MG, Contreras DC, Galvin MB, Gaydorus SS, Holik SD, Jenkins GP, Jones BM, Languell PA, Lyman PJ, March KP, Meuer KA, Peterson SR, Piedmonte MT, Quinn MG, Smaranda NC, Steves PL, Taylor HP, Waddingham TE, Warren JS. Plasma Copper and Zinc Concentration in Individuals with Autism Correlate with Selected Symptom Severity. Nutr Metab Insights 2012;5:41-7.
39. Hoffer A. Children with learning and behavioral disorders. J Orthomol Psychiatry 1976;5:228–230.
40. Russo A, D Robert. Analysis of copper deficiency induces enhanced depression – like and Zinc plasma concentration and the efficacy of zinc therapy in individual with Asperger's syndrome, pervasive developmental disorder not otherwise specified (PDD- NOS) and autism. Biomark insights 2011;6:127-133.

41. Elsheshtawy E, Tobar S, Sherra K, Atallah S, Elkasaby R. Study of some biomarkers in hair of children with autism. Middle East Current Psychiatry 2011; 18:6–10.
42. Geier DA, King PG, Sykes LK, Geier MR. A comprehensive review of mercury provoked autism. Indian J Med Res 2008;128(4):383-411.
43. Jiang HX, Chen LS, Zheng JG, Han S, Tang N, Smith BR.Aluminum-induced effects on Photosystem II photochemistry in citrus leaves assessed by the chlorophyll a fluorescence transient. Tree Physiol. 2008;28(12):1863-71.
44. Naeher LP, Aguilar-Villalobos M, Miller T. Blood lead survey of children, pregnant women, professional drivers, street workers, and office workers in Trujillo, Peru. Arch Environ Health 2004;59(7):359-62.
45. Mohamed Fel B, Zaky EA, El-Sayed AB, Elhossieny RM, Zahra SS, Salah Eldin W, Youssef WY, Khaled RA, Youssef AM. Assessment of Hair Aluminum, Lead, and Mercury in a Sample of Autistic Egyptian Children: Environmental Risk Factors of Heavy Metals in Autism. Behav Neurol 2015;2015:545674.
46. Fang T, Aronson KJ, Campbell LM. Freshwater fish consumption relations with total hair mercury and selenium among women in Eastern China. Arch Environ Contam Toxicol 2012;62(2):323-32.
47. Geier DA, Geier MR. A prospective study of thimerosal containing Rho(D)-immune globulin administration as a risk factor for autistic disorders. J Matern Fetal Neonatal Med 2007;20(5):385-90.
48. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. Trends Neurosci 2008;31(3):137-45.
49. Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. Am J Psychiatry 2006;163 (7): 1290-2.
50. Hardan AY, Jou RJ, Keshavan MS, Varma R, Minshew NJ. Increased frontal cortical folding in autism: a preliminary MRI study. Psychiatry Res 2004; 131 (3): 263-8.
51. Kosinovsky B1, Hermon S, Yoran-Hegesh R, Golomb A, Senecky Y, Goez H, Kramer U. The yield of laboratory investigations in children with infantile autism. J Neural Transm (Vienna) 2005;112: 587-96.
52. Kwon CH, Luikart BW, Powell CM, et al. Pten regulates neuronal arborization and social interaction in mice. Neuron 2006; 50: 377-88.
53. Sato A. mTOR, a Potential Target to Treat Autism Spectrum Disorder. CNS Neurol Disord Drug Targets. 2016;15(5):533-43. Review. PubMed PMID: 27071790
54. Chez MG, Chang M, Krasne V, Coughlan C, Kominsky M, Schwartz A. Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. Epilepsy Behav 2006; 8(1):267-71.
55. Reinhold JA, Molloy CA, Manning-Courtney P. Electroencephalogram abnormalities in children with autism spectrum disorders. J Neurosci Nurs 2005;37 (3):136-8.
56. Gubbay SS, Lobascher M, Kingerlee P (1970) A neurologic appraisal of autistic children: results of a western Australian survey. Dev Med Child Neurol 12:422–429].
57. Ošlejšková H, Dusek L, Makovska A, Rector I. Epilepsia, epileptiform abnormalities, non-right-handedness, hypotonia and severe decreased IQ are associated with language impairment in autism. Epileptic Disord 2007;9(1):S9-18.
58. Amiet C, Gourfinkel-An I, Laurent C, Carayol J, Génin B, Leguern E, Tordjman S, Cohen D. Epilepsy in simplex autism pedigrees is much lower than the rate in multiplex autism pedigrees. Biol Psychiatry 2013;74(3):e3-4.

Table (1): Clinical data of all children

Data	Number	%
Age (years)		
Mean ± SD	6.77±2.45	
Range	3 - 16	
Sex		
Males	228	71.25
Females	92	28.75
Consanguineous marriage	126	39.37
Residence		
Ruler area	211	65.94
Urban area	109	34.06
Family history of Autism	62	19.37
Family history of Epilepsy	46	14.37
Clinical presentation		
Speech delay	133	41.56
Global developmental delay	56	17.5
Decreased socialization	131	40.9
Epilepsy	53	16.56
EEG abnormalities	42	13.12
MRI abnormalities	27	8.43

Table 2: Detailed description of all cases diagnosed as inherited metabolic disorders

	Clinical description	Diagnostic Work-up	Diagnosis	Management
Cases 1,2	13 years old male child and his sister 11 years old, 2nd and 3rd siblings of consanguineous marriage attended to the Pediatric neurology clinic complaining of hyperactivity, mental retardation, and obesity. No history of convulsion. Their physical examination revealed delayed speech and hyperactivity. No other focal neurological abnormalities were noted. Their parents thought a medical advice for looking over the etiology of obesity and mental slowing.	All hormonal profiles were normal. Normal levels of serum ammonia, lactate and normal ABG. Lead, copper, mercury and zinc were within normal values. MRI brain and EEG were normal. The results of the extended metabolic screening and urinary organic acid profile unexpectedly revealed that the two siblings had significantly high phenylalanine level.	PKU	Phenylalanine-restricted diet is started; their plasma phenylalanine level was decreased to a target blood level appropriate for their ages with improvement of hyperactivity and their behavioral attitudes.
Cases 3,4	10 years old male child and his sister 8 years old, 1st and 2nd siblings of consanguineous marriage came to our clinic complaining of impaired reciprocal communication, delayed speech, generalized tonic clonic convulsion. Their physical examination revealed	Normal levels of lead, copper, mercury and zinc. Ammonia, lactate and ABG were normal. MRI brain was normal; EEG revealed generalized epileptogenic activities. Plasma amino acid analysis was	PKU	Clinical improvement was achieved with phenylalanine restricted diet and antiepileptic drugs.

	blonde features, no speech and hyperactivity. No other neurological abnormalities were detected.	significant for phenylalanine with no abnormalities in their urinary organic acids.		
Case 5	4 years old female child, 1st sibling of consanguineous marriage complaining of impaired reciprocal communication, mental retardation and a lack of eye contact that started at 18 months of age. Her physical examination demonstrated black colored skin with no other abnormalities. The parents considered a medical advice to ask about the possibility of having mentally retarded sibling in the next pregnancies.	MRI brain and EEG were normal; serum ammonia, lactate and ABG were normal. Normal trace elements. Plasma amino acid analysis was abundant for phenylalanine with no abnormalities in their urinary organic acids. The patient was diagnosed as PKU. When the mother gets pregnant, the plasma amino acid analysis was performed for her baby who unfortunately diagnosed with PKU.	PKU	Phenylalanine-restricted diet is started in this case and also for her newborn baby in the first month of life.
Case 6	5 years old male child, 2nd sibling of non-consanguineous marriage was complaining about a lack of speech, diminished socialization, and frequent temper tantrums and generalized tonic convulsion started at 3 years of age. His physical examination demonstrated blonde colored skin with no other abnormalities.	Normal levels of lead, copper, mercury and zinc. MRI brain and EEG were normal. Serum ammonia, lactate and ABG were normal. High phenylalanine was detected in plasma amino acid analysis with no abnormalities in his urinary organic acids.	PKU	Phenylalanine restricted diet.
Case 7	5 years old male child, 1st sibling of non-consanguineous marriage was complaining of delayed speech and diminished socialization. The child has received a speech therapy with no much improvement. The mother sought medical advice in our clinic as her younger one month old infant accidentally diagnosed as PKU by the newly established screening program for PKU in Egypt. His physical examination demonstrated no abnormalities.	We asked for the phenylalanine level to all of her siblings. Unfortunately, her older child with delayed speech also had high phenylalanine level. Normal serum ammonia, lactate, ABG and urinary organic acids. MRI brain and EEG were normal. Normal levels of lead, copper, mercury and zinc.	PKU	The patient was referred to the pediatric nutrition clinic to arrange about the phenylalanine restricted diet.
Case	8 years old male patient, 3rd	Normal trace elements.	Glutaric	Oral riboflavin

8	sibling of non-consanguineous marriage was diagnosed with an ASD due delayed speech and difficulty with socialization. He was mentally retarded and exhibited a lack of interest in the surroundings, as well as stereotypical movements. His physical examination revealed no abnormalities.	MRI brain revealed bilateral symmetrical involvement of the subcortical white matter in the frontal lobes, EEG was normal, serum ammonia and lactate were normal while ABG showed compensated metabolic acidosis. Plasma amino acid analysis was normal. His urinary organic acid analysis was significant for increased levels of Glutaric acid and 3-OH-glutaric acid.	aciduria	therapy at a dose of 100 mg/day, oral coenzyme Q 10 at a dose of 100 mg/day and L-carnitine at a dose of 100 mg/kg/day, along with tryptophan-lysine restricted diet.

ABG: arterial blood gases;EEG:electroencephalogram; MRI: magnetic resonance imaging; PKU:phenylketonuria

Table 3: Description of autistic children with trace elements disorders

Data	**Zinc deficiency N=16**	**High Copper N=5**	**High Lead N=2**	**High Mercury N=1**
Age (years)				
Mean ± SD	5.4±1.2	6.4±2	6.3±1.8	5±0
Sex				
Males	9 (56.25%)	3 (60%)	2 (100%)	-
Females	7 (43.75%)	2 (40%)	-	1 (100%)
Trace element levels				
Mean ± SD	55.25±8.03	127.4±9.94	14±1.41	13±0
Reference values	70-153 ug /dl	63.8-110 ug/ dl	<10 µg/dL*	0-9 ng/ml**
Clinical Findings	Decreased socialization (37.5%); Speech delay (25%); Epilepsy (25%); Diarrheal episodes (18.75%); Global developmental delay (12.5%).	Decreased socialization (80 %); Speech delay (20%); Failure to thrive (20%).	Speech delay	Decreased socialization
EEG findings				
Generalized paroxysn	4 (25%)			
MRI findings				
Atrophic changes	3(18.75%)	2(40%)		

*Serum non-toxic lead level; **Serum non-toxic mercury level

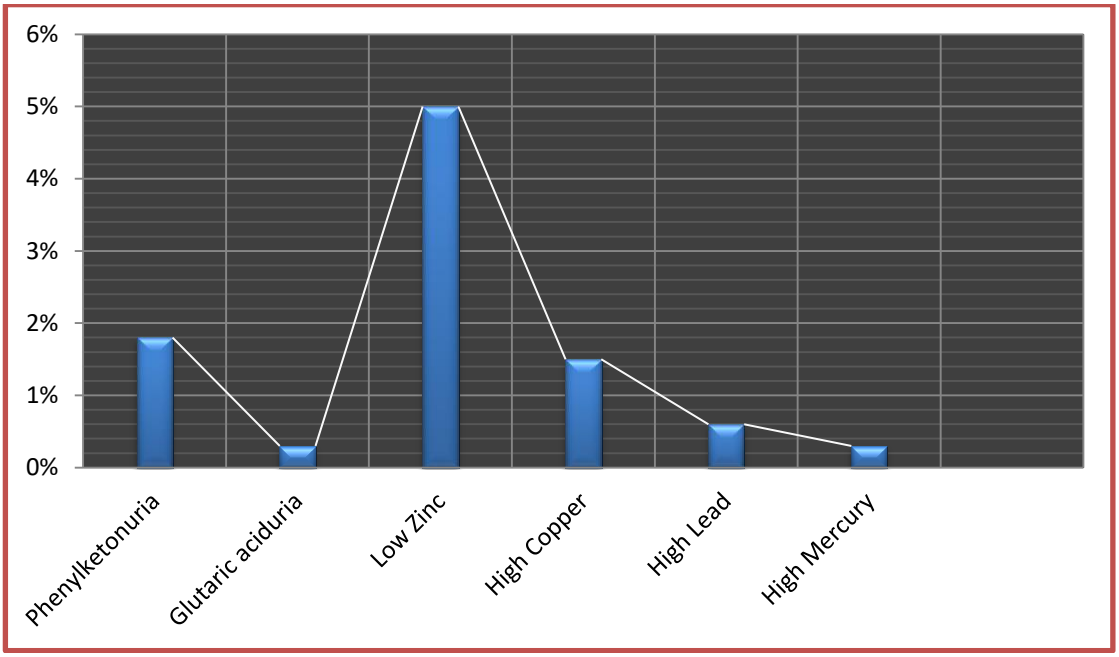

Figure (1): The frequency of inherited metabolic disorders and trace elements disorders in the autistic children