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Plasma Concentrations of the Trace Elements Copper, Zinc, Lead and Selenium in Children with Autistic Spectrum Disorder at Zagazig University Hospitals

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ABSTRACT

Background: Autistic spectrum disorder (ASD) has a multifactorial etiology involving interactions between genes, environment, and diet. Among the environmental factors that have received significant attention related to ASD are toxic metals, such as lead (Pb). Given the importance of zinc (Zn) and copper (Cu) metabolism for healthy neurological functioning and detoxification of heavy metals. Selenium (Se) is an essential trace element having a very narrow range between deficient, essential, and toxic doses.

Objective: To evaluate the association between the level of serum Cu, Zn, Pb, and Se in children with ASD.

Patients and Methods: Our case-control study was carried out in the psychiatric and neurology clinic, Pediatric Department, Zagazig University Hospital, during the period 2018 - 2019. It enrolled 42 children, (13 males and 8 females) with autism diagnosed by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) and the Childhood Autism Rating Scales (CARS) (with no other medical disease). The control group enrolled 21 apparently healthy children.

Results: There was a statistically significant difference between the studied groups regarding the presence of plasma zinc and selenium (lower in the autistic group). There was a statistically significant difference between the studied groups regarding the presence of copper and plasma lead (higher in the autistic group). There was a statistically significant negative correlation between plasma selenium and lead level**.**

Conclusion: Our results suggested an association between serum Cu, Zn, Pb, and Se in children with ASD.

Keywords: Trace Elements, Copper, Zinc, Lead, Selenium, Autistic Spectrum Disorder.

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopment disorder that is present from early childhood and affects daily functioning. The condition is characterized by social-interaction difficulties, communication challenges, and restricted, repetitive patterns of behavior, interests, or activities[(1)]**.**

The number of children with ASD has during the last decades increased at an alarming rate. Currently, it is estimated that 1 in 45 children in the US has ASD diagnosis[(2)]**.** ASD is influenced by both genetic and non-genetical factors[(3)]. Non-genetical factors include immunological, nutritional, metabolic, and environmental factors[(4)].

A significant impact of the interaction between genetic and environmental factors in the etiology of ASD has been demonstrated[(5)].

Essential trace elements are also known to play a significant role in ASD. In particular, zinc (Zn) playing an important physiological role in the modulation of immunity, antioxidant system, was shown to be essential for brain function. Zinc deficiency was found to be frequent in ASD children[(6)]**.** At the same time, copper (Cu) also being an essential micronutrient may have a negative impact on ASD in the case of overexposure. Moreover, certain antagonistic interactions between Zn and Cu are proposed to be involved in ASD pathogenesis[(7)].

The role of other essential trace elements in ASD is less studied. In particular, certain studies have demonstrated altered cobalt (Co)[(8)]**,** selenium (Se)[(9)], manganese (Mn)[(10)]**,** and iron (Fe)[(11)], metabolism in ASD.

This study aimed to evaluate the association between the level of serum Cu, Zn, Pb, and Se in children with ASD.

PATIENTS AND METHODS

This study was carried out in the psychiatric and neurology clinic, Pediatric Department, Zagazig University Hospital, during the period 2018 - 2019.

The present study was designed to be a case-control type. It enrolled 42 children, (13 males and 8 females) with autism diagnosed by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) and the Childhood Autism Rating Scales (CARS) (with no other medical disease). Their ages ranged between 3 to 11 years with a mean of 6.6±2.9 years.

The control group enrolled 21 apparently healthy children, matched to the patients' age and sex. All controls were also clinically examined by the pediatricians to exclude the possibility to have any subclinical autistic features.

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Inclusion criteria:

Children aged from 3-11 years from the pediatric and psychiatric outpatient clinic of, Zagazig University Hospital suffering from autistic spectrum disorder, no previous use of Dimercaptosuccinicacid (DMSA) or other prescription chelators, no anemia or current treatment for iron deficiency anemia, no liver or kidney disease, and children are well hydrated, receiving adequate daily intake of water.

Exclusion criteria:

Other neurodevelopmental disorders sharing common features, mental retardation, abnormal liver &renal functions, nutritional disturbances, medications that affect serum zinc level e.g. captopril and plasma copper level e.g. phenobarbital.

Ethical consent:

Written informed consent was obtained from all participants and the study was approved by the Research Ethics Committee of the Faculty of Medicine, Zagazig University. Studies have been performed on research with human subjects following the Code of Ethics of the World Medical Association (Declaration of Helsinki).

All studied patients have been subjected to the following:

Thorough history taking:

Demographic data (age and sex), height, and weight were obtained. A BMI was calculated. The onset of clinical manifestations, course, and duration. Past history about the presence or absence of perinatal problems. Family history about the presence or absence of psychiatric disorders. Antenatal and maternal history showing the age of the mothers, parity or fetal loss, and medications.

Thorough clinical examination with special emphasis on:

1. **Clinical data of the patients especially the following:** Stereotype and hyperactivity. Eye contact and focus attention. Convulsions. Speech (normal or delayed), expressive and receptive language. Fine and gross motor movement. Tiptoeing movement.
2. **Neurological examination:** Neurological examination was done in the neurology unit in the Neurologic Pediatric Department of Zagazig University Hospital.
3. **Psychiatric evaluation:** Diagnosis of autism using (DSM-IV-TR) criteria of autism[(1)].
4. **Assessment of plasma zinc level** (normal level 63.8 - 110 µg/dl), serum copper (normal level 70- 153 g/dl), serum Selenium (normal level 70-150 ng/m) and serum lead (normal level <10µg/dl).
5. **Specimen collection and storage:** The usual precautions for puncture were observed and followed. Five milliliters of venous blood were drawn from the antecubital vein collected by a syringe with a wide bore needle 5ml. Non-fasting samples were drawn from all participants for estimating plasma Zn, pb, Se level, and serum Cu levels.

Statistical analysis

All data were collected, tabulated, and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA 2011). Quantitative data were expressed as the mean ± SD & median (range), and qualitative data were expressed as absolute frequencies (number) & relative frequencies (percentage).

Independent samples Student's t-test was used to compare between two groups normally distributed variables. While the Mann Whitney U test was used for non-normally distributed variables. Percent of categorical variables were compared using the Chi-square test or Fisher exact test when appropriate. All tests were two-sided. p-value < 0.05 was considered statistically significant (S), and p-value ≥ 0.05 was considered statistically insignificant (NS).

RESULTS

There was a statistically non-significant difference between the studied groups regarding gender, age, or residence. However, there is a significant difference between them regarding the presence of peeling paints **(Table 1).**

Table (1) Comparison between the studied patients regarding demographic characteristics

Demographic characteristics	Studied groups		Test	
	Autism group	Control group	X2/t	p
	N=21 (%)	N=21 (%)		
Gender:				
Male	13 (61.9)	12 (57.1)	1.527	0.217
Female	8 (38.1)	9 (42.9)		
Residence:				
Rural	11 (52.4)	12 (57.1)	0.096	0.757
Urban	10 (47.6)	9 (42.9)		
Peeling paint:				
No	7 (33.3)	18 (85.7)	Fisher	0.001**
Yes	14 (66.7)	3 (14.3)		
Age (year):				
Mean ± SD	8.52 ± 2.14	9.86 ± 2.24	-1.973	0.055
Range	5 – 12	5 – 13		

**p≤0.001 is statistically highly significant

There was a statistically significant difference between the studied groups regarding the presence of stereotypic movements, abnormal speech, absent eye contact, delayed motor movement, and pica. However, there is a non-significant difference between them regarding the presence of convulsions **(Table 2).**

Table (2) Comparison between the studied groups regarding the presenting symptoms

Presenting symptoms	Studied Groups		Test	
	Autism group	Control group	X2/t	p
	N=21 (%)	N=21 (%)		
Stereotypic movement:				
No	3 (14.3)	21 (100)	Fisher	<0.001**
Yes	18 (85.7)	0 (0)		
Absent eye content:				
No	3 (14.3)	21 (100)	Fisher	<0.001**
Yes	18 (85.7)	0 (0)		
Abnormal speech:				
No	0 (0)	21 (100)	Fisher	<0.001**
Yes	21 (100)	0 (0)		
Delayed motor development:				
No	3 (14.3)	21 (100)	Fisher	<0.001**
Yes	18 (85.7)	0 (0)		
Convulsion:				
No	19 (90.5)	21 (100)	Fisher	0.488
Yes	2(9.5)	0 (0)		
Pica:				
No	5 (23.8)	21 (100)	Fisher	<0.001**
Yes	16 (76.2)	0 (0)		

****p≤0.001 is statistically highly significant**

There was a statistically significant difference between the studied groups regarding the presence of plasma Zinc and selenium (higher in the control group), plasma Copper, and lead (higher in the autistic group) **(Table 3).**

Table (3) Distribution of the studied patients according to trace elements

Trace elements	Studied Groups		Test	
	Autism group	Control group	t	p
	N=21 (%)	N=21 (%)		
Zinc (µg/dl) Mean ± SD	70.77± 9.94	114.48 ± 8.93	-15.027	<0.001**
Cupper (µg/dl) Mean ± SD	139.48 ± 14.5	102.76 ± 17.52	7.396	<0.001**
Selenium (ng/dl) Mean ± SD	92.38 ± 4.88	116.67 ± 21.87	-.3.36	0.002*
Lead (PPM): Median	32	17	Z -4.146	<0.001**

**p≤0.001 is statistically highly significant

There was a non-significant difference between the studied groups regarding the sufficiency of Zinc level. However, Zinc deficiency increased the risk of developing autism by 11.06 times**.** There is a significant difference between the studied groups regarding the sufficiency of Copper level. However, excess Copper increased the risk of developing autism by 3.1 times. There is a non-significant difference between the studied groups regarding the sufficiency of Selenium level. However, Selenium deficiency increased the risk of developing autism by 2.24times. There is a significant difference between the studied groups regarding abnormality of Lead level. However, the excess of Lead increased the risk of developing autism by 39.26 times **(Table 4).**

Table (4) Comparison between the studied groups regarding trace elements sufficiency among the studied patients

Trace element sufficiency	Studied Groups		Test		OR (95% CI)
	Autism group	Control group	X^2	P	
	N=21 (%)	N=21 (%)			
Lead: Normal (<10µg/dl) High	0 (0) 21 (100)	4 (19) 17 (81)	Fisher	0.107	11.06 (0.56 –219.7)
Zinc: Deficient Sufficient (70-125)	11 (52.4) 10 (47.6)	0 (0) 21(100)	Fisher	<0.001**	3.1 (1.86 – 5.16)
Cupper: Sufficient (70-140µg/dl) Excess level	10 (47.6) 11 (52.4)	0 (0) 21 (100)	Fisher	<0.001**	39.26(2.1-732.37)
Selenium: Deficiency Sufficient **(70-150ng/ml)**	4 (19) 17 (81)	0 (0) 21 (100)	Fisher	0.107	2.24 (1.57 – 3.18)

**p≤0.001 is statistically highly significant OR Odds ratio CI Confidence interval

There was a statistically significant negative correlation between serum Zinc and both plasma Lead and Copper. However, there is a significant positive correlation between plasma Zinc and Selenium level. There is a statistically significant negative correlation between plasma Copper and both plasma Zinc and Selenium. However, there is a significant positive correlation between it and Lead level. There is a statistically significant negative correlation between plasma Lead and plasma Copper.

However, there is a significant positive correlation between it and both Lead and Selenium levels. There is a statistically significant positive correlation between plasma Lead and plasma Copper. However, there is a significant negative correlation between it and both Lead and Selenium levels. There is a statistically

significant positive correlation between plasma Selenium and plasma Zinc. However, there is a significant negative correlation between it and both Lead and Copper levels **(Table 5).**

Table (5) Correlation between trace elements among the studied patients

Plasma level of	Zinc		Copper		Lead		Selenium	
	r	P	r	P	r	p	r	p
Zinc	1		-0.71	<0.001**	-0.49	<0.001**	0.534	<0.001**
Copper	-0.71	<0.001**	1		0.527	<0.001**	-0.513	<0.001**
Lead	-0.49	<0.001**	0.527	<0.001**	1		-0.42	0.006*
Selenium	0.534	<0.001**	-0.513	<0.001**	-0.42	0.006*	1	

*p<0.05 is statistically significant **p≤0.001 is statistically highly significant r Pearson correlation coefficient

DISCUSSION

In our study, there is a statistically non-significant difference between the studied groups regarding gender. In the present work, 85.7% of autistic children have stereotyping.

In harmony with the present study, **El-Meshad *et al.*** [12] found that, 90% with stereotyped behavior. This agrees also with **El-Baz *et al.*** [13] who found 90% of autistic children have stereotyping which is characteristic of autism and nonspecific eating disorders.

In our study, 9.5% of the patients had a history of convulsions. These findings were compared with the result of a study made by **Blazek**[14] **who** stated that epilepsy is common in autism, with prevalence rates ranging from 7% to 46%.

In our study, 85% of the patients have absent eye contact and all of them (100%) have abnormal speech. This is in accordance with **Klin *et al.*** [15] who stated that children with autism tend to focus on the area around the mouth rather than on the socially informative eye area and on static objects rather than moving people. This agrees also with **El-Meshad *et al.*** [12] who found that, 85% of our patients the condition presented with delayed speech, and 90% with a loss of eye contact.

This study showed that there is a statistically significant difference between the studied groups regarding the presence of plasma Zinc (lower in the autistic group). This was in accordance with that reported by **El-Meshad *et al.***[12] who found that plasma Zn is statistically lower in patients than in controls ($P < 0.001$) as the mean Zn level of children with autism was 68.7 ± 26.4 µg/dl compared with 94.7 ± 11.9 µg/dl in controls. There was a highly statistically significant difference between cases and controls as regards plasma Zn ($P = 0.001$). This is in agreement with the findings of **Faber *et al.***[16], who stated that the frequency of Zn deficiency is high in children diagnosed with ASD, and a study by **Lakshmi and Geetha**[17]**,** in which a significant variation was found for Zn in both hair and nails of low-functioning autism group children when compared with a control group and other study groups

This study showed that there was a statistically significant difference between the studied groups regarding the presence of plasma Selenium (lower in the autistic group). In a cohort of Egyptian children with ASD, the Se intake was lower than that in the healthy controls (7.3 ± 2.0 vs. 8.3±2.3, $p = 0.004$) **Meguid *et al.***[4]**.** In harmony with the present study, **Jory, and McGinnis**[8]**,** found Red blood cell (RBC) Se levels were to decreased in a Canadian sample of autistic children as compared to the controls.

Generally, the obtained data on serum Se levels in ASD patients are in line with the previous findings in blood compartments. In particular, it has been demonstrated that children suffering from ASD are characterized by lower Se levels[18].

This study showed that there is a statistically significant difference between the studied groups regarding the presence of Copper (higher in the autistic group). This agrees with **El-Meshad *et al.***[12] who found that serum Cu was statistically higher in patients than in controls ($P = 0.001$) as the mean Cu level of children with autism was 151.6 ± 54.6 µg/dl compared with 105.4 ± 16.1 µg/dl in controls. This is in agreement also with a study by **Russo and De Vito**[19], who stated that autistic children have significantly elevated plasma levels of Cu ($P = 0.0133$).

In our study, there is a statistically significant difference between the studied groups regarding the presence of plasma lead (higher in the autistic group). Current results were consistent with that of **El-Ansary *et al.*** [20] who demonstrated a significant increase in erythrocyte heavy metal (lead). The existing data demonstrate that environmental exposure to lead has a significant impact on the incidence of ASD. Increased erythrocyte Pb levels were observed in children with ASD.

In particular, **Adams *et al.*** [21] found a significant 41% increase in RBC lead levels in children with ASD.

In the present work, there is a statistically significant negative correlation between plasma selenium and lead level**.** This agrees with **El-Ansary *et al.*** [20] who found a significant negative interaction

between Se and toxic heavy metals, Pb, in children with ASD was revealed in their study. It is well documented that Pb and Se have antagonistic effects. Reduced Se uptake may affect glutathione peroxidase (GPx) activity as Se- dependent enzyme may increase the susceptibility of the cell to oxidative damage. The protective effect of Se against Pb toxicity can be through one or more of three mechanisms. These mechanisms are (i) formation of an inactive Se-Pb complex; (ii) stimulation of radical scavenging through the activation of superoxide dismutase (SOD), thereby increasing the removal of the superoxide radical; and (iii) increasing the antioxidant capacity of cells indirectly by increasing the activity of glutathione reductase, which has an important role in maintaining a sufficient level of GSH in the reduced form. Based on this information, the significantly lower Se/Pb reported in the present study can easily be related to all the oxidative stress-related markers previously reported in patients with ASD[22].

CONCLUSION

Our results suggested an association between serum Cu, Zn, Pb, and Se in children with ASD.

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