Original Article

The Effect of Gender on the Level of Some Salivary Elements in Children with Autism Spectrum Disorder

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Abstract

Background: Autism spectrum disorder (ASD) is a term that refers to a group of neurodevelopmental disorders marked by a lack of social communication and a preference for repetitive activities. **Objectives:** The goal of this research was to determine how gender influenced salivary component levels in children with ASDs. **Materials and Methods:** It included 30 autistic youngsters ranging in age from 7 to 12 years old. All ASD youngsters of both sexes who met the diagnostic criteria for autism had saliva samples obtained. Using a consistent process, all salivary elements were determined using a Flame Atomic Absorption Spectrophotometer by air–acetylene. **Results:** Except for the Cu element, where there was a significant difference between the two investigated groups in this study, there was no significant difference between the two studied groups (male and female autistic children). To the extent of our knowledge, this is the first study to investigate the concentration of many important metallic components in saliva among Iraqi autistic children. **Conclusion:** In children with autism, there is a considerable influence of gender on salivary components.

Keywords: Autism spectrum, salivary elements, salivary metals

Introduction

Autism spectrum disorder (ASD) describes a group of neurodevelopmental diseases usually associated with deficits in social communication and repetitive behaviors.[1] Totally, more than 50 million individuals complained of ASD.[2] This is a lifelong exhausting disorder that load the governments and families of ASD children with extra financial charge.[3] Pathogenesis of this disease is thought to be multifactorial, including genetic factors, deficiency in zinc, deficit in the immune system, toxins, abnormal melatonin synthesis, stress, maternal diabetes, and parental age.[4,5] According to the World Health Organization (2018), ASD affects one out of every 160 children, with males being affected four times more than girls. ASD is more common in Caucasians than in Afro-descendant and Hispanic children. ASD is a heterogeneous behavioral disorder that is characterized by qualitative deficits in social communication and interaction and restricted, repetitive behavioral patterns, activities, and interests.[6,7]

The 0.5–1.5 L of saliva that human salivary glands make each day helps with mastication, swallowing, and speech, lubricates the mouth mucosa, and serves as an aqueous medium for taste perception. By secreting lipases and amylases, they also take part in the digestion of lipids and carbohydrates. Additionally, the numerous organic components in saliva act as a barrier against infections. These include lactoferrin, which sequesters iron (a component necessary for bacterial growth); lysozymes, which cause bacterial agglutination; autolysin, which breaks down bacterial cell walls; and the secretory piece, a glycoprotein that forms a complex with immunoglobulin A (IgA) to defend against viruses and bacteria. Saliva also has ionic components like bicarbonates that act as buffers for bacterially generated acids and shield the esophagus and mouth cavity from gastric juice. Because of this, saliva is essential in defending the mouth against persistent buccal mucosal infections and dental caries.[8]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_466_23

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Submission: 23-Apr-2023 **Accepted:** 09-Aug-2023 **Published:** 29-Mar-2025

How to cite this article: Abdulla WL, Abd ST, Alkhayoun JD, Ghathwan KH, Ali AL. The effect of gender on the level of some salivary elements in children with autism spectrum disorder. Med J Babylon 2025;22:140-5.

Zinc is a fundamental trace element and a remarkable antioxidant; although it is a metalloenzyme that is necessary for the catalytic activity of at least 300 enzymes, but it is not a free radical scavenger. Zinc is found in a large number of transcription factors, significantly more than the known number of Zn-containing enzymes.[9,10] During pregnancy, childhood, and adolescence, zinc is required for optimal growth and development.[8,9] Zn deficiency for a prolonged time may cause impairment in growth.[11] A kid born with a Zn deficit will be at risk for Zn deficiency throughout childhood.[12] In autistic people, changes in gut flora and function are prevalent[13]; for that reason, it is possible that malabsorption of zinc is related to degenerative abnormalities in the mucosa of the intestine that end up as one possible cause of Zn deficiency in patients with autism. During the absorption and utilization process, Zn interacts with other metals such as Cd, Ca, Cu, and Fe. Cd accumulates in high Zn concentrations. Uptake into the duodenum, jejunum, and ileum walls is inhibited.[14]

Copper is a trace element that plays a significant part in redox reactions in live human cells. Ceruloplasmin, a copper-binding antioxidant protein, is responsible for the majority of copper transport in the blood.[15] The human body has between 1.4 and 2.1 µg of copper per kilogram of body weight. Albumin transports copper over the gut wall and into the liver, where it is converted into ceruloplasmin, a copper transport protein.[16]

Lead (Pb) is a well-known neurotoxicant that can impact neurodevelopment by passing across the blood–brain barrier.[17-19] Pb, for example, hinders intellectual development, resulting in behavioral and physical hyperactivity. It can also be harmful to children's health, producing behavioral and neurological issues as well as a drop in intelligence quotient levels.[17,18,20-22] To some extent, the high levels of heavy metal antagonists (e.g., Ca) in the body operate as a protective mechanism. In any case, a Ca deficit can amplify the toxicity of Pb.[19,21,23]

The aim of this study was to estimate the level of lead, copper, zinc, and calcium in the saliva of these patients and evaluating the effect of gender on the level of elements in the saliva of a child with autism estimating the level of lead, copper, zinc, and calcium in the saliva of these patients and evaluating the effect of gender on the level of elements in the saliva of a child with autism.

Material and Methods

Subjects a total of 30 children, this study comprised systemic health patients who had been diagnosed with ASD. The patients' ages varied from seven to twelve years old. Exclusion criteria included any youngster with a history of systemic illness or who was taking any drugs. Five milliliters of unstimulated saliva were collected from each child. Each youngster was asked to rinse his mouth with water to ensure that all debris is removed, then wait 1–2 min for the water to clear. The saliva were collected in a tiny polypropylene tube, and the sample collection was performed early in the morning. The saliva was centrifuged for 10 min at 4000 rpm. The centrifuged supernatants were be kept at (-80°C) until analytical time. Using a consistent process, all salivary elements were determined using a Flame Atomic Absorption Spectrophotometer by air–acetylene.

Statistical analysis

Data was analyzed and computed by using the SPSS (statistical package for social research) SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 694 (including the number and the date on October 11, 2022) to get this approval.

Results

The results of this study are illustrated in the following tables. Table 1 shows descriptive statistics for all salivary elements for 30 autistic children in this study as one group. The unit for Cu, Zn, Mg, Ca, and Cr was µg/dL, while the unit for Na and K was mmol/L.

Table 1: Descriptive statistics: mean and standard deviation for all salivary elements in this study for autistic children

	N	Minimum	Maximum	Mean	Std. deviation
Cu	30	130.000	162.000	152.367	7.393
Zn	30	61.000	81.000	71.400	4.523
Mg	30	1.070	1.560	1.238	0.118
Ca	30	7.700	9.900	8.567	0.593
Na	30	139.000	162.000	149.400	7.815
Cr	30	0.010	0.020	0.014	0.003
K	30	3.100	5.200	3.957	0.646

Table 2: Significance difference between male and female (autistic children) for all salivary elements in this study

	Groups of autistic children	*N*	Mean	Std. deviation	*t*	df	Sig.
Cu	Male	15	149.667	8.616	-2.117	28	0.043
	Female	15	155.067	4.832			
Zn	Male	15	72.000	5.904	0.721	28	0.477
	Female	15	70.800	2.597			
Mg	Male	15	1.263	0.130	1.164	28	0.254
	Female	15	1.213	0.104			
Ca	Male	15	8.740	0.709	1.647	28	0.111
	Female	15	8.393	0.403			
Na	Male	15	147.933	6.563	-1.029	28	0.312
	Female	15	150.867	8.879			
Cr	Male	15	0.015	0.004	1.789	28	0.084
	Female	15	0.013	0.003			
K	Male	15	4.107	0.641	1.287	28	0.209
	Female	15	3.807	0.636			

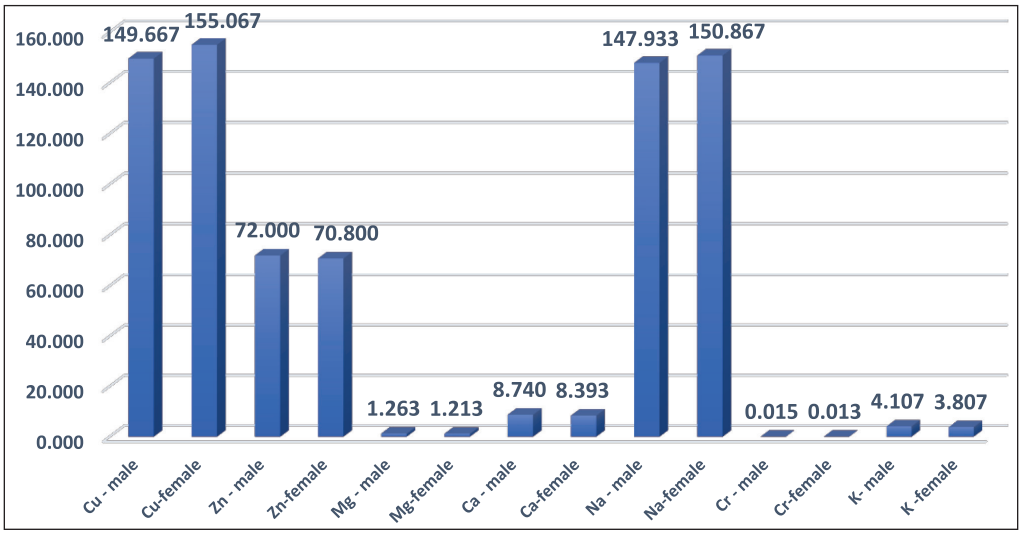

Figure 1: Mean concentration for all salivary elements in autistic children (male and female)

Concerning the significance level, there wasn't any significant difference between the two studied groups (male and female autistic children), except for the Cu element, in which there was a significant difference between the two studied groups in this study, as revealed by Table 2.

Figure 1 shows the mean value for all salivary elements in autistic children (male and female) in this study, according to the units for each measured element. The unit for Cu, Zn, Mg, Ca, and Cr was µg/dL, while the unit for Na and K was mmol/L.

Table 3 illustrates the correlation among all salivary elements in this study; when taken as one group regardless of gender, there was a positive correlation between the numbers of salivary elements, while other elements show a negative correlation. On the other hand, there was no correlation between some salivary elements (Na with Zn, Ca, and K with Cu, Zn, Mg, and Cr).

The correlations between each salivary element in male autistic children are revealed in Table 4; there wasn't any correlation between Na or K with all other salivary elements, while other correlations were almost positive or negative.

The correlations between each salivary element in female autistic children are shown in Table 5.

Discussion

The prevalence of ASD is increasing in the world, with an estimate of 1/68–1/50 per individual. Early diagnosis and treatment of such diseases enhance the functional abilities of those children.[24]

To the extent of our knowledge, this is the first study to investigate the concentration of many important metallic components in saliva among Iraqi autistic children.

Salivary diagnostics is a promising field in the diagnosis and monitoring of the disease process. There is increased

Table 3: Correlations between all salivary metals in autistic children (male and female)

		Zn	Mg	Ca	Na	Cr	K	
Cu	*r*	-0.521(**)	-0.616(**)	-0.657(**)	0.481(**)	-0.670(**)	-0.028	
	P	0.003	0.000	0.000	0.007	0.000	0.882	
Zn	*r*	1	0.538(**)	0.713(**)	-0.201	0.532(**)	0.216	
	P		0.002	0.000	0.287	0.002	0.251	
Mg	*r*		1	0.504(**)	-0.644(**)	0.709(**)	-0.153	
	P			0.005	0.000	0.000	0.418	
Ca	*r*			1	-0.061	0.666(**)	0.431(*)	
	P				0.749	0.000	0.017	
Na	*r*				1	-0.597(**)	0.510(**)	
	P					0.000	0.004	
Cr	*r*					1	-0.234	
	P						0.213	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 4: Correlations between all salivary metals in male autistic children

		Zn	Mg	Ca	Na	Cr	K	
Cu	*r*	-0.534(*)	-0.485	-0.655(**)	0.366	-0.520(*)	-0.063	
	P	0.041	0.067	0.008	0.18	0.047	0.824	
Zn	*r*	1	0.682(**)	0.667(**)	-0.356	0.736(**)	-0.006	
	P		0.005	0.007	0.193	0.002	0.984	
Mg	*r*		1	0.672(**)	-0.323	0.605(*)	0.096	
	P			0.006	0.24	0.017	0.734	
Ca	*r*			1	-0.177	0.868(**)	0.257	
	P				0.527	0.000	0.355	
Na	*r*				1	-0.496	0.418	
	P					0.06	0.121	
Cr	*r*					1	-0.09	
	P						0.75	

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 5: Correlations between all salivary metals in female autistic children

		Zn	Mg	Ca	Na	Cr	K	
Cu	*r*	-0.437	-0.855(**)	-0.495	0.663(**)	-0.891(**)	0.304	
	P	0.103	0.000	0.06	0.007	0.000	0.27	
Zn	*r*	1	0.151	0.907(**)	0.042	-0.008	0.688(**)	
	P		0.592	0.000	0.881	0.979	0.005	
Mg	*r*		1	0.048	-0.955(**)	0.825(**)	-0.610(*)	
	P			0.864	0.000	0.000	0.016	
Ca	*r*			1	0.22	0.144	0.661(**)	
	P				0.432	0.607	0.007	
Na	*r*				1	-0.686(**)	0.716(**)	
	P					0.005	0.003	
Cr	*r*					1	-0.646(**)	
	P						0.009	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

attention toward the role of metallic components in ASD occurrence. The wide distribution of toxic chemicals and metals are more have a tendency to be associated with the etiology of ASD.[25]

To screen through important metal elements for cell function, zinc is the second most abundant element in the human body, and it is necessary for cellular activity. Zinc is present in 10-fold in the brain compared to body serum level, which reflects the importance of this element in neurodevelopment.[26]

Previous studies indicate zinc shortage causes aberrant neural tube closure in animal models.[27]

There are many studies in the literature that evaluate the level of zinc in different body fluids (blood, urine, serum) and hair follicles of children with autism, but very few studies evaluate the salivary zinc concentration in those children.

The results of this study showed that the level of zinc in ASD children was with a minimum value of 61.000 µg/dL and a maximum value of 81.000 µg/dL with an average of 71.400 µg/dL. This is a very important indicator for deficiency or low levels of zinc in ASD children in our study. Regarding gender, there was no significant difference between male and female (autistic children) in the concentration of zinc in saliva.

This is in coincidence with a study conducted by Deshpande *et al.*[24], estimated the level of zinc in Unstimulated entire saliva samples taken from 10 autistic and 10 healthy youngsters of mixed dentition age. The minimum level of zinc in ASD children was 7.02 mmol/L and the maximum 11.86 mmol/ L, while the level of zinc in healthy children was a minimum value of 15.44 mmol/L and a maximum value of 23.92 mmol/L. They illustrated the low level of zinc in saliva, which reflects the important role of zinc in the development of this disease.

In this study, the level of copper in ASD children had a minimum value of 130.000 and a maximum value of 162.000 with a median of 152.367. Regarding gender, there was a significant difference between males and females (autistic children) in the concentration of copper in saliva.

A study by Li *et al.*[28] investigated the concentration of copper and zinc in the serum of children with ASD. They discovered that ASD children's mean serum zinc levels and Zn/Cu ratio were much lower than normal children's, whereas serum Cu levels were significantly greater.

They conclude an association between serum levels of Zn and Cu and ASD among Chinese patients, and the Zn/Cu ratio could be considered a biomarker of ASD[29]. A median concentration of calcium in the whole saliva is 1.23 mMol/L. Therefore, individuals with calcium levels below the median were considered as "low," while those with higher levels were considered as "high" salivary calcium individuals.

The level of calcium in ASD children was with a minimum concentration of 7.700 µg/dL and a maximum level of 9.900 µg/dL with a median of 8.567 µg/dL. This means that the calcium level in ASD children is slightly higher than normal levels as compared to the standard level of calcium in healthy individuals. There was no significant difference in Ca level between the male and female groups. Normal concentrations of salivary Na^+ range from 8.7 to 24 mEq/1 (average 17.4 mEq/1). Also, salivary K+ levels should be between 13 and 16 mEq/1, with an average of 14.1 mEq/1.[30] The level of sodium in the saliva of ASD children was with a minimum value of 139.000 and a maximum value of 162.000 with a median of 149.400.

Conclusion

With continuous advances in the medical field, the study found that salivary biomarkers for the diagnosis of autism at an early age as this will be a dramatic change in the disease treatment protocol. The findings of the current study demonstrated that salivary Cu level could be used as an indicator for this disorder. Because of the paucity of clinical studies on this subject, more studies are needed in the future to reach to a real clue about this subject.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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