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RESEARCH

The Assessment of Selenium, Aluminum, and Zinc in Children with Autism Spectrum Disorder

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Abstract

ASD is a complex condition defined by many causes, one of them being excessive concentrations of necessary and harmful chemicals in children. The serum, hair, and nails of children with ASD have lower levels of critical trace elements, according to studies. It is quite obvious that bio elements are involved in physiology and pathophysiology. Thus, this study examined trace element contents in serum samples from children with autism spectrum disorder (ASD), specifically zinc (Zn), aluminum (Al), and selenium (Se). The study also looked for links between trace element levels and autistic severity. The study included 47 children with autism spectrum disorder, and the Gilliam's Scale was used for severity. The study also included 53 healthy kids with age and gender-matched with those of ASD. For serum trace element analysis, graphite furnace atomic absorption spectrophotometry was used. The study found significant decreases in selenium and zinc concentration (OR, 5.25; CI, 1.96 ~ 14.08; $p < 0.001$) and increases in aluminum level (OR, 39.34; CI, 8.20 ~ 89.45; $p < 0.001$) in children with ASD compared to the control group. The area under the curve (AUC) values of 0.85 for Se, 0.98 for Al, and 0.7 for Zn showed high sensitivity and specificity for all parameters. Results indicate a strong positive connection between ASD and their levels of selenium (Se) and zinc (Zn) (β, 0.48; CI, 0.280 ~ 0.679; $p < 0.001$ and β, 0.31; CI, 0.10 ~ 0.52; $p = 0.005$). There is a negative correlation between ASD and aluminum (Al) (β 0.83; CI, 0.71 ~ 0.95; $p < 0.001$). This element may be a biomarker for autism in youngsters. High odds ratio (OR) values indicate trace element risk in autistic children.

Keywords Autistic · Selenium · Aluminum · Zinc

Introduction

ASD is a disability that is identified based on observable behaviors, which of difficulties in social communication, social interaction, and impairment in language. Individuals with autism also exhibit a limited range of interests and frequently engage in repetitive behaviors and mannerisms [1]. Autism spectrum disorder (ASD) was initially documented in 1943, and afterward, there has been a notable surge in the prevalence of ASD on a global scale [2]. Despite extensive research and analysis spanning several decades, the precise etiology of autism remains elusive. This is mostly due to the complex and varied nature of the condition, which manifests with variable degrees of severity [3]. The incidence of ASD has seen a notable rise in recent years. According to recent data, the prevalence of ASD in the USA was found to be one in 54 children aged 8 years across 11 different sites. Additionally, the incidence of ASD was seen to be 4.3 times higher in males compared to girls. In China, the prevalence of ASD among children aged 6 to 12 is estimated to be 0.7%. These findings are based on studies conducted in the respective regions [4, 5]. The escalating incidence of ASD has engendered apprehensions within the realm of public health. The precise etiology of ASD remains uncertain; nonetheless, it is widely acknowledged that the development of ASD is influenced by a combination of genetic and environmental variables [6]. Research has shown that an imbalance of trace elements can lead to impairments in the nervous system and biological dysfunctions linked with (ASD) [7]. Zinc (Zn), copper (Cu), and selenium (Se) are essential trace elements that play crucial roles in multiple physiological functions

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and are needed for various enzymatic processes. Therefore, the biochemical regulation of these elements is of utmost importance [8]. Previous studies have noted that the antagonistic interactions between zinc (Zn) and copper (Cu) can have a significant impact on the pathophysiology of ASD by influencing the metallothionein system, which in turn leads to the induction of cellular excitotoxicity [9]. Zinc can detoxify heavy metals if interacts with them increasing the synthesis of metallothionein. The heavy metals compete with copper and zinc for binding with metallothionein because of having a high affinity for binding with it [10]. Selenium is a crucial component of numerous antioxidant enzymes, such as thioredoxin reductase, iodothyronine deiodinase, and glutathione peroxidase. Selenium plays a vital antioxidant role in the regular activities of humans and shields cells from the damaging effects of free radicals as a component of enzymes that catalyze oxidation and reduction reactions [11]. Selenium can exert its detoxification effect on mycotoxin poisoning by reducing reactive oxygen species production and mitochondrial dysfunction, enhancing cell viability and function, and inhibiting the immune response [12]. Also, Se can further counteract the immunotoxin effects of T-2 toxins on T lymphocytes [13]. Se can detoxify heavy metals such as cadmium, inorganic mercury, methylmercury, thallium, and to a limited extent silver, depending on the selenium/mineral interaction. If the selenide combines with the heavy metal, ions will be given a metal selenide, which is metabolically inert [14]. Additional trace metals arsenic (As), lead (Pb), aluminum (Al), and cadmium (Cd) are known to have a significant impact on autism spectrum disorder (ASD) by triggering neuroinflammation, oxidative stress, excitotoxicity, and disrupting the balance of critical ions within the body. Furthermore, the elements manganese (Mn) and iron (Fe) have been found to potentially contribute to neurotoxicity, which may have a significant impact on the development of autism spectrum disorder (ASD) [15]. Numerous studies have been conducted to investigate the presence of essential and hazardous elements in children diagnosed with ASD. However, the predominant focus of these investigations has been on the analysis of trace element concentrations in blood or hair [16–18]. Previous studies have observed a correlation between trace element concentrations and the presence of social communication difficulties and repetitive behaviors in children with ASD as compared to typically developing children [19]. In addition, individuals who have received a diagnosis of ASD may manifest a range of characteristic behaviors associated with the condition, such as challenges in communication, repetitive actions, self-harming tendencies, disturbances in sleep patterns, and difficulties in adhering to dietary norms [20]. Furthermore, it is important to note that the manifestation of these autistic symptoms might exhibit significant variability across individuals [21]. Numerous studies have been conducted to evaluate the association between ASD and the levels of trace elements [22]. Therefore, it is essential to acknowledge the significance of evaluating the association between ASD and blood trace elements as a viable approach for the early detection of the condition, because is cost-effective.

Study Design

The current study comprised two cohorts. The first cohort consisted of children diagnosed with autism spectrum disorder (ASD) at the Hamaem Al-Salam Center in Iraq/Najaf, totaling 47 participants. The second cohort served as the control group and included 53 individuals. Furthermore, the study exclusively focused on children diagnosed with autism spectrum disorder (ASD), while children with other mental disorders were deliberately excluded from the research. It is noteworthy to remark that the control samples were obtained from youngsters residing in the Karbala province of Iraq. The Gillim scale was employed to assess the severity of ASD in the individuals. Ultimately, the age range of participants in both groups spanned from 3 to 12 years of age (mean ± SD, 6.35 ± 2.37).

Results

Characteristic Features

The questionnaire is completed by the parents of the children or psychological trainers employed at the autism center who oversee the care of children diagnosed with autism. From the same children, the blood samples were collected in order to assess their clinical characteristics. The aforementioned questions have been incorporated into the compilation of questions as presented in Table 1.

Distribution of Samples

After performing the distribution test (Kolmogorov-Smirnov and Shapiro-Wilk test) for the samples, it was found that they had an abnormal distribution (p-value for all elements less than 0.05), as illustrated in Figure 1. In light of the results of this test, the most effective strategy for carrying out statistical procedures was selected.

Blood Parameters Under Study

The concentrations of three trace elements, namely selenium, aluminum, and zinc, were measured in the serum specimens of all participants. A comparison was then conducted between the two groups involved in the study, and the results are displayed in Table 2. Interestingly, a

Table 1 Clinical characteristics

Sample characteristics	Children with ASD, *N* (%)	Healthy children, *N* (%)
Sex		
Male	31 (66)	36 (67.9)
Female	16 (34)	17 (32.1)
Age (y): mean ± SD	6.35 ± 2.37	6.30 ± 2.4
ASD severity level		—
Medium	28 (60)	
Under medium	19 (40)	
Hyperactivity		—
Yes	27 (57)	
No	20 (43)	
Living		
Villages and countryside	5 (11)	14 (26.4)
City	42 (89)	39 (73.6)
Lack of oxygen at birth	Non	—
Talking		
Speaking	22 (47)	50 (94.3)
Non-speaking	25 (53)	3 (5.7)
Family history		—
Hereditary		
YES	8 (17)	
NO	39 (83	
Father's academic achievement		
Collegiate	25 (53)	9 (17)
Secondary	10 (21)	10 (18.9)
Primary	8 (17)	12 (22.6)
Reads and writes	4 (9)	22 (41.5)
Mother's academic achievement		
Collegiate	28 (60)	5 (9.4)
Secondary	5 (11)	13 (24.5)
Primary	11 (23)	18 (34)
Reads and writes	3 (6)	17 (32.1)
Father's job		
Employee	33 (70)	23 (43)
Earner	14 (30)	30 (57)
Mother's job		
Employee	39 (83)	13 (24.5)
Housewife	8 (17)	40 (75.5)

significant decrease in the levels of selenium and zinc elements and a significant increase in the levels of aluminum were observed in children with autism spectrum disorder (ASD). Specifically, the odds ratios (OR) and confidence intervals (CI) for selenium were found to be 5.25 (CI, 1.96~14.08; $p < 0.001$), for aluminum 39.34 (CI, 8.20~89.45; $p < 0.001$), and for zinc 3.75 (CI, 1.44~9.76; $p = 0.02$).

Spearman's correlation coefficient (r) test was used to examine the correlation between elements measured. Through results presented in Table 3, noted a strong negative only between selenium and aluminum ($p < 0.001$).

The ROC analysis test is the basic measure of accuracy: sensitivity (the probability the diagnostic test is positive for disease for a patient who truly has the disease) and specificity (the probability the diagnostic test is negative for disease for a patient who truly does not have the disease) [23]. It was utilized to calculate the area under the curve for all parameters (Figure 2) and considered an excellent value (AUC of Se, 0.85; AUC of Al, 0.98; and AUC of Zn, 0.70). The optimal cutoff values, sensitivity, and specificity for serum trace elements are presented in Table 4. The percentage sensitivity and specificity for all trace elements under this study are considered excellent pedigree.

Method

Blood Specimen Collection

Venous blood samples, measuring 5 mL each, were obtained from both cohorts, consisting of individuals with (ASD) and a control group. Subsequently, the specimens were transferred into gel tubes and allowed to clot at ambient temperature. Following clotting, the samples were subjected to centrifugation with a force of 3000 times the acceleration due to gravity (xg) for 10 min. The serum was isolated and afterward stored in an Eppendorf tube at a temperature of −20 °C ready for the next step [24].

Determination of Trace Elements

The furnace atomic absorption spectrometry (AAS) (SHIMADZU AA-6300\Japan) technique with low detection limits for 0.2 ppb located in the laboratories of the College of Medicine, University of Karbala was used to determine trace elements in environmental and biological samples by conversion of an element into a free atomic state by means other than flame, such as using electronic furnaces and then measuring the absorbed light of a certain wavelength in the ground state.

The maxim wavelength for all elements is determined by plotting absorbance vs. wavelength in the graph. Moreover, the absorbance maximum is used instead of some other point on the absorption curve because the maximum is the most reliable position to measure. The wavelength for selenium was 196 nm for aluminum (309.3nm) and 213.9 nm for zinc.

Ethical Approval Declarations

The study involved the collection of blood samples from children with autism, following the necessary administrative procedures, including obtaining consent from parents. This

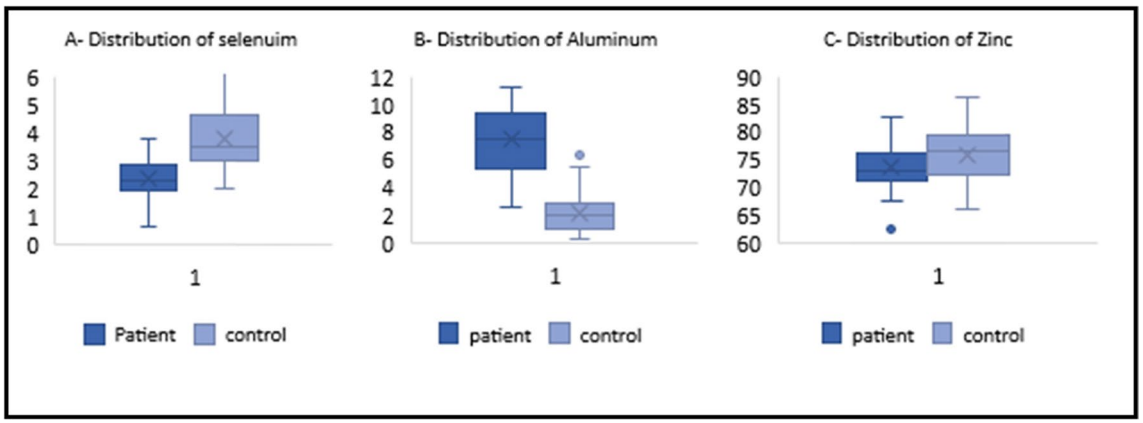

Fig. 1 Box-whisker plots of serum selenium, aluminum, and zinc levels in children with ASD and healthy group

Table 2 The concentration of trace elements under study between children with ASD and healthy control

Parameters	Children with ASD ($N=47$), median (range)	Healthy group ($N=53$), median (range)	OR	CI (95%)	p-value
Selenium (µg\L)	2.26 (3.11)	3.17 (4.61)	5.25	1.96 ~ 14.08	< 0.001
Aluminum (µg\L)	7.57 (8.61)	2.08 (5.95)	7.68	2.64 ~ 22.34	< 0.001
Zinc (µg\L)	73.13 (20.35)	76.55 (20.11)	3.75	1.44 ~ 9.76	0.02

N number of samples, *ASD* autism spectrum disorder, *OR* odd ratio, *CI* confidence interval 95%

Table 3 The correlation between trace elements understudy in children with ASD

		Zinc	Aluminum
Selenium	*r*	0.117	−0.593**
	Sig	0.109	< 0.001
Aluminum	*r*	0.140	–-
	Sig	0.200	

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

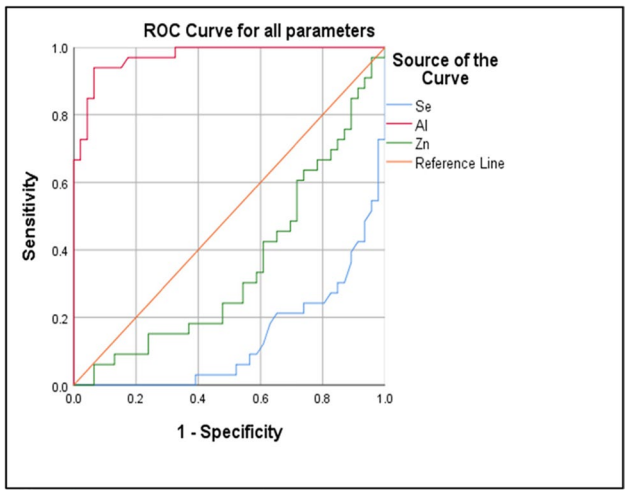

Fig. 2 ROC analyses of trace element understudy

process was conducted under the supervision of the Dean of the College of Science and the Head of the Chemistry Department at the University of Kerbala, with oversight provided by the professor overseeing the research. The specimens were obtained from the participants at the central site situated in Najaf, Iraq. Furthermore, the Karbala University Ethics Committee process was followed in a current project under the form number 6633257/KUEC.

Statistical Analysis

The data results were tested using version 26 of the Statistical Package for the Social Sciences (SPSS), developed by IBM. The Shapiro test, which is a normality test, was employed to assess the distribution of the samples. The Shapiro-Wilk test revealed a violation of the assumption of normality, thus necessitating the utilization of the Mann-Whitney *U* test to analyze the data gathered in this study. The receiver operating characteristic (ROC) analysis test was employed to compute the area under the curve, which is widely regarded as a reliable measure of sensitivity and specificity in detecting and identifying. On the other hand, a linear regression model was employed to examine the association between serum selenium (Se), aluminum (Al), and

Table 4 Coordinates of ROC curve for serum selenium, aluminum, and zinc discriminate ASD

	Selenium	Aluminum	Zinc
AUC	0.85	0.98	0.70
Cutoff value	3.12	4.01	76.94
Sensitivity %	87%	95%	78%
Specificity %	61%	85%	58%
CI 95%	0.792 ~ 5.05	3.66 ~ 23.339	2.615 ~ 8.327
PPV %	61%	85%	60%
NPV %	73%	94%	70%
Accuracy	70%	91%	65%

AUC area under the curve, *PPV* positive productive value, *NPV* negative productive value, *CI* confidence interval 95%

zinc (Zn) levels and autism spectrum disorder (ASD). To examine the relationship between the variables in this study, a correlation analysis was conducted. The statistical analysis in this study utilized Spearman's rho correlation coefficient (r). A p-value of 0.05 or below was deemed to be statistically significant in all conducted statistical tests.

Discussion

The results of this study suggest a notable correlation between reduced selenium levels and the occurrence of autistic spectrum disorder. Selenium, along with its related selenoproteins, plays a crucial role in various biological processes, including the manufacture of thyroid hormones, DNA replication, reproductive capacity, and antioxidant defense [25]. Selenium (Se) plays a role in immune function by modulating the activity of activated T lymphocytes. Multiple studies have demonstrated that the administration of selenium (Se) supplements results in an accelerated and enhanced immune response [26]. The vital role of Se in brain function continues to be apparent. Selenoprotein P (SEPP1) is accountable for the transportation of selenium (Se) to the brain and serves a neuroprotective function in mitigating oxidative stress. Mice lacking the selenoprotein P1 (SEPP1) gene display evident impairments in brain function. The mice in this study had brain damage and eventual mortality as a result of insufficient dietary selenium [27]. The majority of selenoproteins have a role in the cellular response to oxidative stress [28], and the ability to counteract oxidative stress is crucial for proper neurodevelopment. The occurrence of heightened oxidative stress has been commonly documented in children diagnosed with ASD [29]. This oxidative stress is mostly attributed to the presence of reactive oxygen species (ROS) [30], elevated levels of lipid peroxidation [31], and diminished concentrations of antioxidants [32]. It is noteworthy that a particular antioxidant, glutathione peroxidase-1 (GPx1), which is a selenoprotein and recognized as a gene associated with ASD, regularly exhibits lower levels in children diagnosed with ASD [33]. In recent studies, it has been demonstrated that Se can inhibit a specific cellular mechanism known as ferroptosis, which is triggered by oxidative stress [34]. While the investigation of ferroptosis has predominantly focused on its occurrence in adult individuals with stroke, traumatic brain injury (TBI), and Parkinson's disease, there is a possibility that this mechanism also plays a role in nervous development. Therefore, Se deficiency may lead to heightened neuronal susceptibility to oxidative stress in children diagnosed with ASD. Even though Se is essential for healthy brain function, Se neurotoxicity has received a lot of attention lately [35]. Numerous studies have been conducted, especially in the past few years, that indicate different inorganic and organic selenium compounds may have neurotoxic consequences if they are present at spur nutritional levels [36]. All things considered, everyone agrees that inorganic Se species selenite in particular are more neurotoxic than organic Se compounds [37].

The potential association between aluminum (Al) and ASD has been examined by multiple researchers [38, 39]. Despite the prevalence of aluminum in the natural environment, it lacks any discernible biological role within the human body. Numerous studies have demonstrated that aluminum has the potential to induce developmental and immune impairments, disturb hormonal balance, exhibit neurotoxic properties, and impact cognitive functions and behavioral patterns. Aluminum (Al) is a neurotoxin that has been empirically proven to have detrimental effects on the human nervous system for several decades [40]. Previous studies have provided evidence indicating that individuals diagnosed with ASD exhibit a notable presence of aluminum (Al) deposition inside the brain [41]. A study conducted by Melendez et al. demonstrated a correlation between elevated body aluminum levels and the presence of behavioral abnormalities in individuals with ASD [42]. Furthermore, empirical investigations have shown evidence of the capacity of Al compounds to negatively impact social behavior [43]. While there is currently no available evidence suggesting a direct link between aluminum (Al) exposure and catatonia, several researchers have presented findings that support the detrimental impact of Al on motor function [44, 45].

The findings of this study revealed a statistically significant reduction in zinc levels in children diagnosed with ASD. The presence of zinc deficiency can have various impacts on the immunological and nervous systems of the human body. In both the developmental and adult stages of neurogenesis, Zn^{2+} is an essential component for several processes such as proliferation, migration, differentiation, and survival. Neurons that are deficient in zinc exhibit reduced proliferation, differentiation, and apoptotic pathway activation [46]. In fact, data suggests that the hippocampal

region is most likely the most vulnerable to a zinc deficit [47], which reduces progenitor cell number and neuronal differentiation, thus causing irreversible impairment of learning and memory capacity during early development [48]. There is a suggestion that chronic zinc shortage may lead to reduced efficiency of the adaptive immune system and increased reliance on the innate immune system, even though the innate immune system also experiences damage in the presence of zinc deficiency [49]. According to a study conducted by Bonaventura et al. in 2015, it has been documented that zinc possesses anti-inflammatory properties. Additionally, a zinc deficiency has been found to elevate the levels of pro-inflammatory cytokines and enhance the activity of the central nervous system inflammasome [50]. Zinc has been found to protect against maternal insult induced by lipopolysaccharide in animal models. This protection has been observed in the context of preventing aberrant behavior in object recognition tasks and abnormal sickness behavior following immune challenges. Furthermore, recent studies have shown that zinc can also prevent communication deficits in a mouse model of autism [51]. A recent review has also examined the relationship between zinc and the nervous system [52]. Considering the various functions of zinc, it seems advantageous to examine its classification into three overarching categories: structural roles, cellular signaling, and enzymatic co-factors. Zinc serves as an essential co-factor for many enzymes involved in DNA and RNA polymerization, histone modification, and DNA repair through the action of DNA ligase. Zinc plays a crucial role in various facets of protein synthesis inside the central nervous system (CNS) and functions as an autonomous factor in regulating gene expression [53]. In recent times, zinc has been recognized as a crucial constituent in structural proteins, particularly in the context of zinc-finger motifs [43]. Proteins with widespread presence are frequently responsible for the composition of receptors found in the brain, such as estrogen, thyroid hormone, and glucocorticoid receptors [54]. Zinc plays a crucial role in facilitating the folding process and the subsequent development of the functional structure of these receptors [55]. Zinc has been associated with the development of olfactory, cerebellar, and hippocampus regions, and even a slight shortage of zinc has been demonstrated to impact memory and learning [56]. Previous studies have provided evidence indicating that temporary zinc deficit during gestation can have long-lasting effects on memory and learning abilities that extend into adulthood [57].

Conclusion

The findings of this study indicate a correlation between the levels of selenium (Se), aluminum (Al), and zinc (Zn) in serum and autism spectrum disorder (ASD) among patients in Iraq. Additionally, the high percentage of sensitivity and specificity observed for all trace elements examined in this study suggests their potential as reliable biomarkers for diagnosing autism in children. Elevated odds ratio values are indicative of an increased risk of trace element exposure in children diagnosed with autism.

Author Contribution Study design and conception, proofreading, and statistical analysis by Narjis Hadi Al-Saadi. Material preparation, data collection, analysis, and writing—first draft by Ali Fadheel Hamoud. Interpretation of the results by Narjis Hadi Al-Saadi and Ali Fadheel Hamoud.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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