Academic Science Journal

Measuring Iron Concentration in the Blood Samples of Some Autistic Children from Baghdad City

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Received: 20 December 2023 **Accepted: 28 January 2024**

Published: January 2025

DOI: https://dx.doi.org/10.24237/ASJ.03.01.838B

Abstract

Autism spectrum disorder (ASD) research delved into the correlation between genetic susceptibility and various environmental factors, including diet, drug exposure, and environmental toxicants such as exposure to heavy metals. Iron is one of the heavy metals around which there is much controversy regarding their role as neurotoxins in the development of autism, its excess in the brain may cause Alzheimer's disease or Parkinson's disease, while iron deficiency in the blood may increase levels of other heavy metals in the blood and brain, such as cadmium and lead. This study aimed to measure iron levels in samples of autistic children in Baghdad City. Blood serum samples were collected from 60 patients with autism spectrum disorder and 35 healthy controls to measure iron levels. The samples were frozen and analyzed using flame atomic absorption spectrometry. The study found that children with autism had significantly higher iron levels than healthy children with a mean value of 37.409±5.969 µg/ dl ($P \leq 0.05$). Possible causes for this condition include specific drugs, genetic disorders, or the dietary habits of the ASD. Iron may be a factor in ASD, and Iraqi environmental pollution may be the cause of the higher iron levels in autistic children. The study suggests that any change in iron levels might have an impact on the brain since it is an important component of the body. Without a doubt, toxic metal overload or key element shortages can cause epigenetic

modifications that impair neuronal maturation and lead to neurodevelopmental abnormalities in the form of developmental disorders in children.

Keywords: Autism, Heavy metals, iron, Flame atomic absorption spectrometry.

Introduction

Autism spectrum disorder (ASD) is a developmental disability that can be diagnosed. Asperger syndrome, childhood disintegrative disorder, autism spectrum disorder, and pervasive developmental disability not otherwise defined are the four main disorders [1].People with ASD experience the following health problems: challenges with repetitive and self-stimulating activities; and trouble in social contact and communication, sensory difficulties, including extreme sensitivity to or insensitivity to particular auditory, visual, and tactile stimuli, are frequently experienced by people with autism spectrum conditions. These symptoms restrict the sufferers' capacity to interact and engage with their surroundings [2,3]. However, these warning indicators might not materialize, eye contact, facial expression, and body posture problems are just a few examples of how social interactions are affected [4].

Heavy metals are metallic elements with a relatively high density compared to water [5]. Environmental contamination by heavy metals is a growing concern for the environment and public health. This is due to the increasing use of heavy metals in industry, agriculture, and households, which results in more frequent exposure of people to them. In addition to being a naturally occurring mineral in many foods, iron is also sold as supplements. It is a crucial component of hemoglobin, a red blood cell protein that transports oxygen from the lungs to the body's tissues [6]. Iron aids in the maintenance of healthy connective tissue and muscle metabolism as a component of myoglobin, another protein that carries oxygen [7]. In addition, iron is necessary for cell function, cognitive development, physical growth, and the synthesis of many hormones [8]. The best sources of heme iron in our diet are lean meat and shellfish [9]. Fortified grain products, nuts, legumes, and vegetables are dietary sources of nonheme iron. In the US, bread, cereal, and other grain items provide around half of the dietary iron [10]. Breast milk contains highly bioavailable iron but in amounts that are not sufficient to meet the needs of infants older than 4 to 6 months. In the brain tissue of people with dementia from diseases including Alzheimer's (AD), Parkinson's (PD), Huntington's (HD), and Down syndrome (DS),

ferritin, an iron-storing protein, is found in high concentrations [11, 12]. However, little is known about the brain's processes for iron deposition and regional selectivity. Therefore, the quantity of iron in the brain may be determined by looking at the ferritin levels in the cerebrospinal fluid. In cultured systems, glial cell ferritin synthesis [13] and secretion [14] rely on cellular iron levels. Cerebrospinal fluid ferritin levels are regarded to be a good indicator of brain iron levels and can be helpful in clinical circumstances. Restless legs syndrome, a disorder brought on by low brain iron levels and treated with iron supplements, results in decreased CSF ferritin levels [15]. The study aimed to measure the concentration of iron as one of the heavy metals in the blood samples of autistic children from Baghdad City and to correlate these levels with disease severity.

Material and Methods

In this study autism spectrum disorder groups were composed of patients who attended Medical City Hospital for Mental and Psychological Diseases in Baghdad, a specialized center for the treatment of autism, and a private clinic, in addition to patients who were reached through advertising by social media or from acquaintances and friends. The study included patients who live in Baghdad/ Iraq, they were diagnosed by a psychiatric and neurological consultant. Their ages ranged between 3- 15 years and the duration of the disease was different. All patients underwent a cognitive assessment with psychological tests in the psychological counseling department of the hospital. As well as creating a consent form for each patient to participate in the research. Patients with mental, neurological, or inflammatory diseases, or with a history of immunological or malignant diseases, were excluded. Additional exclusion criteria were the use of institutional-related drugs with psychotropic substances, including psychosis and family history. The control group consisted of healthy participants; their ages ranged between 3-15 years. The selected children had no previous family history of ASD or any mental or neurological diseases. Likewise, the same laboratory tests that were performed on the patients were also performed for the participants to compare the results.

Collection of blood samples

Blood samples were collected from 60 patients and 35 healthy children. Five to ten millilitre of blood samples were obtained by vein puncture for each person using a disposable syringe of 10

ml. The blood samples were placed in gel tubes of 6 ml and allowed to clot at room temperature. The serum was then separated from the blood cells by centrifugation at 3000 rpm for 10 minutes. The serum was then transferred to airtight plain tubes and stored in the freezer at -20°C until it was used to measure heavy metal levels [16].

Preparation of standard solutions

The preparation of standard solutions before injection into the flame atomic absorption spectrometry was required to assess the concentration of heavy metals in the blood serum samples and serve as a means of dilution and dissolution [17]. Among these solutions are matrix modifier solutions and calibration solutions.

• Modifiers for the matrix

Triton 100 (10%), ammonium dihydrogen phosphate (20%), and concentrated nitric acid are all present in the homogeneous solution (HNO_3) [18]. 10% of Triton X-100 was prepared by placing 10 ml in a 100 ml volumetric flask or 5 ml of the substance in a 50 ml volumetric flask, followed by deionized water to fill the remaining volume. To prepare 20% of ammonium dihydrogen phosphate, either 20 g of the substance was placed in a volumetric flask with 100 ml of water, which was then filled with deionized water, or 5 g of the substance was placed in a volumetric flask with 25 ml of water, which was also filled with deionized water. One ml of concentrated nitric acid was added to 400 ml of deionized water and 25 ml of triton -100 (10%), as well as 5 ml of ammonium dihydrogen phosphate (20%). Deionized water was added to the volume, and the mixture was thoroughly agitated to ensure that all of the components inside the volumetric flask was homogeneous. The temperature was kept at 18 °C following the preparation of the solution.

• Calibration solution

This solution was prepared by taking a volumetric flask (10 ml). Withdraw 0.5 ml from the serum sample and place it in the volumetric flask, completing the volume with a modified matrix solution.

Measurement of iron in the blood serum

Serum samples were collected from 60 patients suffering from ASD disease and 35 healthy controls that were pre-screened and frozen to measure the concentration of iron (Fe). Iron was

measured using Flame Atomic Absorption Spectrometry (NOV-AA 800/ Analytik Jena /Germany). It is a technique for measuring quantities of chemical elements present in environmental and biological samples by measuring the absorbed radiation by the chemical element of interest. There is more than one method for estimating the flame atomic absorption spectrometry of the elements, and they differ according to the type and concentration of the element to be estimated in the sample [19]. Iron concentration measurement was conducted in the laboratories of the Quality Control Department/ Ministry of Trade. The Statistical Analysis System- SAS program was used to detect the effect of different groups (patients and control) on study parameters [20]. To clarify differences between the means, the least significant differences (LSD) values were calculated at $P \leq 0.01$ and expressed as mean ± standard error (Mean ± S.E).

Ethical Clearance

Consent was obtained from the legal guardians or parents of all participating children. A detailed explanation of the study, its purpose, and benefits, and consent was obtained from the guardians or parents before any data collection. Also, the participation in the study was entirely voluntary, and the children were not subjected to any coercion or undue influence. Parents or guardians were given the freedom to withdraw their children from the study at any point without consequences.

Results

Measurement of the concentration of iron in blood specimens

The iron levels in the blood of patients with ASD compared to healthy control children are represented in Table 1. In the case of the total iron content in the blood, the highest value was recorded in autism spectrum disorder patients at 190.3 µg/ dl, and the lowest value at 6.6 µg/ dl, while 28.2 µg/ dl and 5 µg/ dl were recorded as the higher and lowest values, respectively in the healthy controls. It was found that the mean values of iron concentration in ADS patients reached 37.409± 5.969 µg/ dl, as they were statistically more than the mean values in healthy controls 14.113 ±1.137 µg/ dl as shown in Table 1.

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Table 1: The iron concentration of autism spectrum disorder blood compared with the control

Groups	Iron concentration (µg/ dl)		
	Min.- Max. value	**Mean ± SE**	**Acceptable limit**
Patients	6.6 - 190.3	37.409±5.969	50-60
Control	5- 28.2	14.113 ±1.137	
LSD P≤ 0.05	0.000 S		
	(P≤ 0.05), Significant.		

According to (WHO) and (CDC), the acceptable value for total iron in blood serum is 50-60 µg/ dl [21, 22]. The current study revealed a highly significant difference in iron levels at $P \leq 0.01$ in autistic patients compared to the control, this may be due to the use of nutritional supplements recommended by their physicians (Figure1).

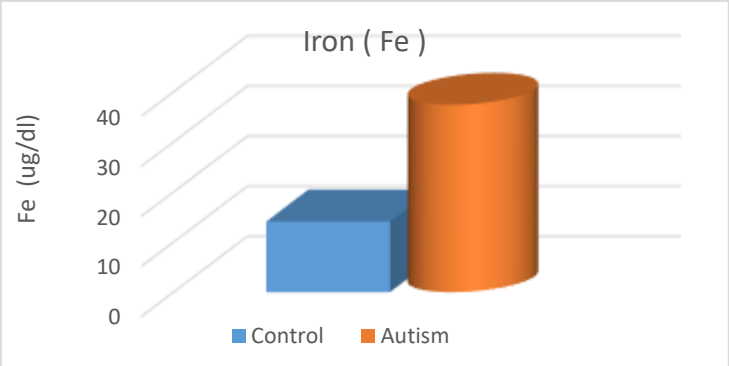

Figure 1: Comparison between the concentration of iron in the blood of ADS patients and control groups

The results of the present study are in agreement with Baj *et al* (2021) concluded that anemia was found only in 1 %–15 % of children with ASD, and the studies did not always provide sufficient markers for iron deficiency diagnosis. As an iron-storing protein, ferritin saturation and transferrin are also often employed markers in anemia studies in addition to hemoglobin (Hgb) levels [23]. In several research on autism, serum ferritin is the most widely utilized iron measure. A low ferritin level is an indicator of iron shortage and a risk factor for iron deficiency anemia [24]. Frequent low ferritin levels impact the dopaminergic system in children with ASD, which may be directly linked to the pathophysiology of the disorder. However, iron supplementation may cause serum ferritin levels to recover to normal. Notably, children with ASD had decreased serum ferritin levels (7–52%) [25]. Iron deficiency anemia is a difficult

diagnosis to make, requiring the assessment of iron status using many markers [26]. Numerous study teams have made an effort to ascertain whether iron insufficiency is highly prevalent in children with ASD and whether iron status in the body and iron consumption are related [27]. As many as 40–50% of pregnancies are affected by iron insufficiency, which is the most prevalent micronutrient deficiency worldwide, according to the WHO. According to the Centers for Disease Control and Prevention (2002) [28]., age-dependent iron levels should be 10 μg/L for children under 6 years old and 12 μg/L for children 6 years old and above to properly interpret the results of the biochemical tests. Nonetheless, some research indicates that there is no correlation between ASD and iron deficiency based on different statistical methods [29]. According to the findings of the meta-analysis conducted by Pao-Yen Lin *et al*. on data up until 2017, there are no appreciable differences between the ASD and non-ASD groups in peripheral iron levels, serum ferritin, Fe in hair, or iron uptake with diet. Inadequate iron levels prevent proper cellular activity in newborns and young children, which is a serious nutritional health risk. Around the world, 47% of children are affected, 50% of them are in poor nations [30], and just 6-12% of them are in wealthy nations [31, 32]. A clinical sample of children with global developmental delay had a higher prevalence of iron deficiency anemia than the overall population. In the brain's homeostasis, development and embryogenesis, immunological modulation (including the immune system), antioxidation, antiapoptotic, neural differentiation, and gene regulation, iron has a unique role [33]. Young children with Autism Spectrum Disorders often have restricted diets and are finicky eaters, which increases their risk of malnutrition [34].

Conclusion

Iron is a vital nutrient for human health, but too much or too little iron can be harmful. Iron helps the body carry oxygen, produce energy, and grow new cells. However, iron can also be toxic if it is not properly regulated. This study found that children with autism had higher levels of iron overload than children without autism. The increased iron levels in children with autism may be due to certain medications, nutritional supplements, or diet. The study suggests that any change in iron levels might have an impact on the brain since it is a vital component of the body. Without a doubt, toxic metal overload or key element shortages can cause epigenetic

modifications that impair neuronal maturation and lead to neurodevelopmental abnormalities in the form of developmental disorders in young children. Autism's pathophysiology is still not entirely understood. It is not advised to treat or supplement with iron for children with ASD without tracking laboratory test results and documenting iron status parameters, since this could result in adverse effects.

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