Concentration of zinc and lead in the blood of patients with autism and their relationship with disease accurrence in Thi-Qar province, south of Iraq.

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Abstract:

The aim of the present study was to evaluate the levels of heavy elements (zinc and lead) in blood of autistic patients to demonstrate the disease's association with these elements.

This study was conducted on (60) autistic patients, aged (1-15) years, in the city of Al-Nasiriyah, Dhi Qar province, southern Iraq. Patient and sample data were collected during 2019, from the Autism Center in the Health Department in Dhi Qar Governorate, the official center in the city and another community center.

The results showed that the heavy elements examined can be one of the causes of the disease, which increases the appearance of symptoms of the disease. The concentrations of all these elements were high compared to control and were at the next level (zinc 106 μg/dL, lead 13.2 μg/dL).

As well as the incidence of sex (5: 1) males: females, and may be the cause of the high rate of infection in males compared to females might be due to sex–affected or from environmental impacts and hormonal effects.

Keywords: Autism, Autistic spectrum disorders, Heavy elements Thi-Qar City-Iraq.

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1.1: Introduction:

Autism is a complex multifactorial epidemic disorders develops due to genetic mutations (1), so by studying a large number of children around the world showed that many resons causes this disorder, such as environmental exposures to heavy elements (2). There is a strong probability that most children who become autistic, because of their inability to produce glutathione. This may have been a fundamental reason. Low glutathione prevents the brain from detoxification of chemicals, among them heavy elements which received from diverse sources (3).

The prevalence of autistic spectrum disorders (ASD) has increased significantly in countries around the world develops due to genetic mutations that delete glutathione or inhibit its development, environmental factors (4) and genetic factors (5,6). High exposures to heavy elements, with glutathione deficiency cause

disruption and interference of brain function, and autism spectrum disorders therefore, detoxification is essential when it comes to getting rid of autism (7,8).

With the increase of pollution resulting from the industries and of transportation, especially in urban areas, and with the continued use of traditional treatments. moreover, the preparations the cosmetic and unhealthy habits increasing the problem of heavy elements poisoning (9).

Pollution in the environment and in the air in particular causes many diseases for humans and congenital malformations. This pollution is the result of the negative development of industry and agriculture and many biological applications that produce many pollutants such as iron plants, chemical industries, energy production, oil refining plants and the use of insecticides in insect control (10). It is possible to cause gene mutations. Heavy elements divided into two types that harm to human and elements that benefit to human in small quantities, as they enter the structure and internal structure of the cells, but if they are taken in large quantities, on the desired limit will affect the health of the patient in the event of a lot of changes in the basement level of the cells, and these elements affect the nervous system and many changes occur on the nerve cells accumulation of these elements within the nervous system during the transmission with the blood, will cause a lot of diseases in the device nervousness, including autism, when children with autism were measuring their blood will find some difference in the concentrations of these elements from their non-infected counterparts. Their presence prevents the brain from producing glutathione. Non-production of glutathione due to gene mutation or because of incontinence may prevent brain cells from the accumulated toxins thus also prevent the brain from producing glutathione. Strengthens the immune system, strengthens its functions, prevents oxidation in cells, fights the presence of free radicals of cells, purifies blood from toxins (11). The pollution by heavy elements can occur by many different ways, directly or indirectly, may contaminate soils, water and plants by material from the air or by direct deposition of pollutants "generally humans are exposed to these metals by ingestion or inhalation (12). Because of their contact with human life and other living organisms, these dangerous and important pollutants are interest to many researchers, and have been a high number of mortality, because of their toxicity (13). Some of heavy elements are beneficial to humans and other living organisms where intervention in the physiological and chemical processes (14).

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Materials and methods:

About, 80 samples of blood were collected from children infected by autism ranging in age from (1.5 to 15) years. The samples were divided into two groups: 60 patients with autism and 20 non-infected samples as control group. Important information was also recorded including age, Gender and period of symptoms.

After obtaining the required information for the child, the blood extraction area was sterilized with a mild disinfectant, and blood was withdrawn from the radial vein. 8 ml of blood was obtained and placed in a test tube (EDTA tube or Jel tube. All tube transported in cool box.

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Measurement of Blood Parameters:

Blood parameters (WBCs, RBCs and Hb and) were measured by hematological analyzer according to manufacturer′s procedure.

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Preparation of serum:

About 3 ml of blood was centrifuged (3000 rpm/minute) for 20 minutes, then serum kept at 20 0c until the digestion for metals values estimation.

Determination of Heavy Element in Blood:

The wet digestion procedure was used (24). For heavy element analysis, 0.5 ml of the blood sample was predigested with 10 ml 1:1 concentrated HNO_3 and $HClO_4$ acids on a hot plate at 135°C until the liquor had finished undergoing oxidation. Thereafter, 5 ml H_2O_2 was added and the temperature was maintained at 135°C for 1 hour until the liquor got completely digested and showed a clear colour. At the end of complete digestion, the digests blood was cooled and subsequently filtered through Whattman (No. 98) filter paper into 100 ml volumetric flask and made up to mark with deionized water by (FAAS).

Statistical analysis:

All data of the current study were statistically analysis by use Microsoft excel version 2010 and spss version 22 by use independent t test for p. value (<0.05).

Results:

1: Distribution of Autistic Syndrome According to Age Group

The results recorded that the high prevalence autistic patients were in male 85% when compared with female 15 with non-significant difference at p. value (≤ 0.05). Also the results showed that the predominate age group was male second age group with 38.3%, while the lowest age group was female third age group was 1.7% as in table (4-1).

Table (1): **Distribution of Autistic Syndrome According to Age Groups**

Groups	Male		Female		Total	
	No.	%	No.	%	No.	%
Below 6 years	19	31.7	5	8.30	24	40.0
6 – 10 years	23	38.3	3	5.00	26	43.3
Above 10 years	9	15.0	1	1.70	10	16.7
Total	51	85.0	9	15.0	60	100%
Calx² = 1.08	**Tabx² = 5.99**		**Df= 2**		**P. Value= 0.58**	

2: Level of Hemoglobin in Autistic patients and Control:

The results show the highest level of hemoglobin in the third age group of patients, with level 13.1 ± 2.2, but the level of hemoglobin in Autistic patients in the outher groups and Control were with normal value. These results also show that there are non-significant differences at ($p \leq 0.05$) in the levels of Hb between age groups of Autistic patients and Control.

Table (2): **Level of Hemoglobin in Autistic patients and Control**

Parameter / Groups		No.	Hb gm/dl Mean ± SD	P. Value
Group 1	Patients	24	12.5 ± 1.4ª	0.28
	Control	7	11.8 ± 1.9 ª	
Group 2	Patients	26	12.8 ± 1.9 ª	0.38
	Control	6	11.9 ± 1.9 ª	
Group 3	Patients	10	13.1 ± 2.2 ª	0.40
	Control	7	12.2 ± 1.6 ª	

3: Level of RBCs in Autistic patients and Control:

The results record the highest level of RBCs in the third age group of patients, with level 4.6 ± 0.46, but the level of RBCs in Autistic patients and Control with normal value.

These results also show that there are non-significant differences at ($p \leq 0.05$) in the levels of RBCs between age groups of Autistic patients and Control.

Table (3): **Level of RBCs in Autistic patients and Control**

Parameter / Groups		No.	RBC $*10^6$/ml Mean ± SD	P. Value
Group 1	Patients	24	4.2 ± 0.66 ª	0.209
	Control	7	4.5 ± 0.38 ª	
Group 2	Patients	26	4.4 ± 0.55 ª	0.38
	Control	6	4.6 ± 0.36 ª	
Group 3	Patients	10	4.6 ± 0.46 ª	0.50
	Control	7	4.5 ± 0.31 ª	

4: Level of WBCs in Autistic patients and Control:

The results recorded the highest level of WBCs in the second age group of patients, with level 11.0 ± 3.4, but the level of WBCs in Autistic patients with abnormal level with except third age group and control with normal value. These results also shows that there are significant differences at($p \leq 0.05$) in the levels of WBCs between age groups of Autistic patients and Control, with except third age group there are non-significant difference between patients and control.

Table (4): Level of WBCs in Autistic patients and Control

Parameter Groups		No.	WBC $*10^3$/ml Mean ± SD	P. Value
Group 1	Patients	24	11.3 ± 3.1[b]	0.022
	Control	7	8.20 ± 2.3 [a]	
Group 2	Patients	26	11.0 ± 3.4[b]	0.05
	Control	6	8.18 ± 1.6 [a]	
Group 3	Patients	10	8.65 ± 3.1 [a]	0.84
	Control	7	8.39 ± 1.5 [a]	

Note: the different letters with sub scribe refer to significant differences

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5: Level of Zn in Autistic patients and Control

The results record the high level of Zn in the first age group of patients, with level 106.0 ± 21.2.

These results also show that there are significant differences at ($p \leq 0.05$) in the levels of Zn between age groups of Autistic patients and Control.

Table (5): Level of Zn in Autistic patients and Control

Parameter Groups		No.	Zn Mean ± SD	P. Value
Group 1	Patients	24	106.0 ± 21.2[a]	0.079
	Control	7	90.3 ± 15.30[a]	
Group 2	Patients	26	92.0 ± 21.7[a]	0.11
	Control	6	77.1 ± 11.7[a]	
Group 3	Patients	10	91.6 ± 17.7[a]	0.18
	Control	7	81.1 ± 10.8[a]	

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6: Level of Pb in Autistic patients and Control

The results show the highest level of Pb in the third age group, with level 13.2 ± 4.82 and second age group with level 12.4 ± 3.77 of Autistic patients. While the low level in the first age group of control with level 3.35 ± 0.83.

These results also show that there are significant differences at ($p \leq 0.05$) in the Pb levels between age group Autistic patients and control.

Table (6): Level of Pb in Autistic patients and Control

Parameter / Groups		No.	Pb Mean ± SD	P. Value
Group 1	Patients	24	9.87 ± 4.00^b	0.001
	Control	7	3.35 ± 0.83^a	
Group 2	Patients	26	12.4 ± 3.77^b	0.005
	Control	6	7.53 ± 1.52^a	
Group 3	Patients	10	13.2 ± 4.82^b	0.006
	Control	7	7.13 ± 1.60^a	

Legand: as in table (4)

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Discussion:

The result of current study recorded that the high prevalence autistic patients were in male 85% when compared with female 15 %, the ratio was about (5:1 male – females). This approach with the most performed studies, which include ratio at (4:1 males- females) (5), but different with study was ratio conducted in Riyadh, Saudi Arabia, recored (15:1 males- females) (15). Furthermore, this study revealed that there was a significant difference according to sex status. This difference in the ratio between sex , can be due to the different genetic makeup of the sex (6) , and some other sex- Influenced traits or the difference about the world ratio may be due to some people classified this disease under psychology disease . Some parents may be not recoded their data (especially   of females) for the center autism.

The results show the highest level of (Pb, Zn) of Autistic patients compared with control. The result of this study showed high level of the Pb element in the autism patients compared with control. The other study showed that 97% of patients have highest level of the Pb element, which is higher than the permissible limit of children when their blood concentration reaches 10 µg/dL (16). Other study there was no significant difference of blood lead (5.68±2.44) (17).

This difference between the results obtaind in our study and those repoting an increase excretion of lead in children with ASD may be also due to genetic and nongenetic factors. Lead finds its way to individuals through food and ambient air as a result of the burning of the fucl containing the element as it is discharged from the car exhaust as well in industrial areas, around mines and oil refineries (18). Lead is a toxic and harmful element on children and other creatures even at low concentrations. It affects the growth and development of the central nervous system (19).

Lead poisoning have deleterious effects on the development of brain areas including these implicated in cognition, communication and social functioning also anther elements have deleterious effects on of the brain in differenced ratio depending to concentration, condition of element (1).

In children, the major sources of lead exposure are lead paint, dust, soil, food and beverage, traditional folk remedies, and parental occupational lead exposure (20). The risk of lead exposure is higher in foetuses

and young children than in adults for several reasons (21). The developing nervous system of children is more sensitive to lead toxicity than the developed brain of adults, and the brain of children aged ≤ 5 years is particularly susceptible to exposure to lead circulating in the blood (22). Prenatal and early childhood lead exposure can cause cognitive and language dysfunction that may persist throughout childhood and even into adulthood (23).

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