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Research Article

Toxic Effect of Some Heavy Metals in Egyptian Autistic Children

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ABSTRACT

Despite the great number of studies being made concerning cellular and the molecular dysfunctions associated with autism, the basic central mechanism of these disorders has not been proposed in the major scientific literature. We suggest that environmental heavy metals which have neurotoxic properties such as lead, arsenic, mercury, cadmium and aluminum can exacerbate the pathological and clinical problems leading to autism. So this study aimed at exploring these possible associations that exist between autism and these neurotoxic heavy metal and which of them has positive correlation with the degree of severity of the disease. Our study comprised two groups. The first one included 55 autistic child (male and female). The second group consisted of 75 normal child (male and female).The two groups where matched for age and gender and socioeconomic status. The results of this study revealed that the mean level of the measured heavy metals were higher among the group suffering from autism compared to their controls. The differences were statistically significant. Also our study reported that the mean levels of the studied metals were the highest among those with sever autism compared to moderate and mild autism. However the difference were not statistically significant except for that of aluminum ($P<0.001$). Also there was positive correlation between the measured heavy metals and severity autism according to CARS. However the positive correlation was statistically significant only for aluminum. So we recommend a novel way of treatment for autistic children including adding much nutritional fibers as psilium husk and bran to food of autistic children to decrease absorption of these metals and or chemical chelation of these heavy metals and this will improve the symptoms of the disease or may treat it, with special emphasis to sources of aluminium environmental pollution.

Key words : aluminium; cadmium; arsenic; lead; mercury; heavy metals autism; CARS; environmental pollution.

INTRODUCTION

Autism is thought to be a multi-factorial disorder, with disruption of the normal neurobiologic mechanisms and is widely recognized to have a strong genetic component, probably involving multiple gene loci[1]. Autism is a serious neurodevelopmental disorder characterized by impairments in social interaction, verbal and nonverbal communication, and other restricted behaviors. The number of children reported with autistic spectrum disorders (ASDs) has increased dramatically during the last 10 years, but it is difficult to determine how much of this increase represents actual incidence and how much may be due to increased awareness and diagnosis. First estimated to occur in 4 to 5 per 10,000 children, the incidence of autism is now 1 per 110 in the United States, and 1 per 64 in the United Kingdom, with similar incidences throughout the world. The causes remain largely unknown[2-5].

Autism is believed to result from disruption of normal neurobiologic mechanisms primarily in the prenatal period and is widely recognized to have a strong genetic component, probably involving multiple gene loci. Nongenetic factors are also likely involved and may explain some of the increased prevalence. Medications such as thalidomide and valproic acid in utero have been linked to cases of autism[6]. Maternal smoking during pregnancy has also been associated[7] and there are case reports of children with both fetal alcohol syndrome and autism[8]. Other exogenous exposures known or suspected to interfere with neurodevelopment may also play a role in ASD etiology. Heavy metals such as lead and mercury have been relatively well studied in relation to impaired neurodevelopment[9,-12] but few studies have examined associations with autism. Progress in identifying environmental factors which increase autism risk has been made recently[13,14].

The present study aimed at exploring possible associations between some known neurotoxic heavy metals and severity of autism among Egyptian autistic children to uncover environmental causes of autism especially in Egypt.

SUBJECTS AND METHODS

Subjects

**Author for Correspondence*

Table 1 : General characteristics of the studied groups

Parameter	Autism (n=55)	Control (n=75)	Test of significance	P-value
Age (years) - Mean ± SD	4.01 ± 1.91	4.02 ±1.33	t = 0.25	> 0.05
Gender				
Male (No and %)	39 (70.9%)	57 (76%)	$\chi 2$ = 0.426	> 0.05
Female (No and %)	16 (29.1%)	18 (24%)		
CARS (Mean ± SD)	35.45 ± 3.75	----------		

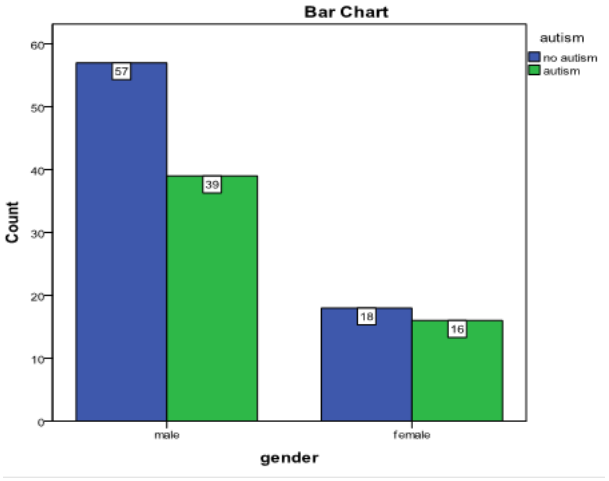

Figure 1: Sex distribution among autistic children and control group

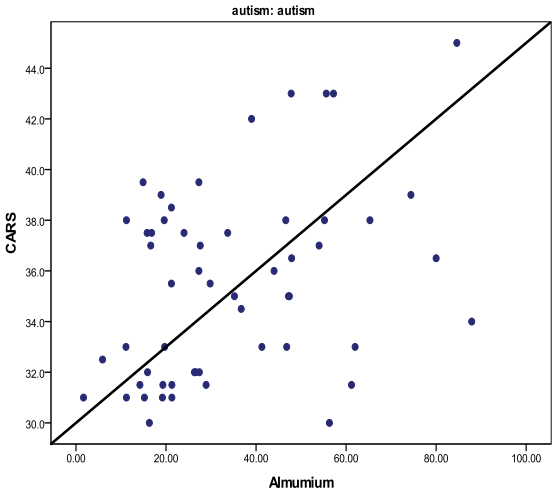

Figure 2: Correlation coefficient between the level of almunium and CARS

This study is a case control, cross sectional study. The cases were 55 autistic child who were randomly selected. They were diagnosed by DSM-V and classified according CARS (Childhood Autism Rating Scale) with a mean age (4.56 ± 2.1) years. And the control were 75 child. The cases and the control were selected from the clinic of the department of Research on Children with Special Needs (Medical division, National Research Center, Cairo, Egypt). Both groups were comparable as regards age and gender and socioeconomic status.

Methods

Standards and reagents

The standards for ICP-MS were prepared from stock solutions of mercury, lead, cadmium, arsenic and aluminum at 10 mg/L concentrations obtained from Sigma- Aldrich, Australia, and labelled as Fluka TraceCert Ultra. Spiked solutions were prepared from the stock as necessary. Ultrapure nitric acid (HNO3) and hydrochloric acid (HCl) reagents were obtained from J.T. Baker Inc. All other reagents and solvents used in this study were analytical grade obtained from Sigma-Aldrich, Australia. All water used in washing laboratory glassware, other apparatus, and in the preparation of sample and standard solutions were deionized (resistance < 18 m_, Academic Milli-Q Ultra Pure Water System, Australia).

Calibration

The ICP-MS was calibrated, using aqueous standard solutions (of the same acid concentration as in the samples and additional internal standards) prepared from the stock solutions by subsequent dilution in the range of 0.05 to 10 mg/l for each element. A mixed working solution containing 10 mg/l of each element is prepared first, as described above, from which the final standard solutions are made.

Analysis of Mercury, Lead, Arsenic, Cadmium and Aluminum

The operating conditions of the ICP-MS instrument were optimized before the analysis was performed. In particular, the nebulizer gas flow rate, the ion lens voltage(s) and the ICP RF power were adjusted to yield the highest signal intensities possible while maintaining low levels of oxides and doubly-charged ion production (both should be less than ~ 3%). After the instrument had been optimized, appropriate calibration standards were then measured. As part of the quality assurance protocol, at least six-point calibrations of different ranges (0.10 to 15.0 mg/L and 0.05 to 2000 µg/L) were carried out for Hg,Pb,As, Cd and AL to generate a calibration curve with correlation coefficient of 0.999 or better. The sample solution concentrations were then determined from the corresponding calibration curve. Calibration standards were also analyzed at regular intervals during analytical runs for the ICP-MS to ensure that the instrument continued to meet acceptable sensitivity and linearity criteria.

All "Potentially Toxic Elements" are reported as µg/g creatinine; all other elements are reported as µg/mg creatinine. Normalization per creatinine reduces the potentially great margin of error which can be introduced by variation in the sample volume. It should be noted, however, that creatinine excretion can vary significantly within an individual over the course of a day[15].

Statistical analysis

SPSS package program was used for statistical analysis. Cases and control subjects were compared by using the

Table 2 : Mean and Standard deviation of the measured parameters among the studied groups

Parameters	Autism (n=55)	Control (n=75)	Test of significance	P-value
	Mean ± SD	Mean ± SD		
- Mercury (ug/g cr)	11.03± 6.63	2.22±0.55	t = 11.47	<0.01**
- Lead [a.] (ug/g cr)	12.47 ±17.56	3.6 ± 1.04	z= 3.59	<0.01**
- Arsenic (ug/g cr)	175.6 ± 160.65	74.44 ± 31.44	t = 5.32	<0.01**
- Cadmium [a.] (ug/g cr)	1.81± 2.61	1.32 ± 0.54	z = 3.35	<0.05*
- Aluminum (ug/g cr)	34.56 ± 20.88	17.56 ± 5.75	t = 6.72	<0.01**

*p<0.05 ** p<0.01 [a.] =Mann Whiteny test

Table 3: Mean and Standard deviation of the measured parameters according to severity of autism.

Parameters	Mild Autism (n=31)	Moderate Autism (n=19)	Severe Autism (n=5)	ANOVA	P-value
	Mean ± SD	Mean ± SD	Mean ± SD		
Mercury (ug/g cr)	9.62 ± 6.51	11.64 ± 6.93	12.66 ±5.20	0.701	>0.05
Lead (ug/g cr)	9.21 ± 15.29	12.89 ± 17.55	22.24 ± 25.12	1.114	>0.05
Arsenic (ug/g cr)	172 ± 192.09	156.67 ± 142.91	306.60 ± 69.97	1.948	>0.05
Cadmium (ug/g cr)	1.75 ± 2.79	1.86 ± 2.28	2.03 ± 3.17	0. .031	>0.05
Aluminum (ug/g cr)	30.51 ± 19.03	35.32 ± 21.73	56.84 ± 17.12	3.801	<0.05*

*p<0.05

Student's t test for numeric variables.
No normal distribution of both lead and Cadmium was observed for the exposed group so we used non parametric tests (Mann Whiteny) test.

RESULTS

Our study revealed that there was no statistical significant differences between autistic children and control group as regards age and sex and socioeconomic status (table 1). This is illustrated in figure 1.

It is quiet apparent from table 2 that the mean level of the measured heavy metals were higher among the group suffering from autism compared to their controls. The differences were statistically significant.

It is quite evident from table 3. that the mean levels of the studied metals were the highest among those with sever autism compared to moderate and mild autism. However the difference were not statistically significant except for that of aluminum (P<0.001).

Table 4 classify that there was positive correlation between the measured heavy metals and severity autism according to CARS and figure 2 shows that there were positive correlation between mean levels of the studied metals and CARS. However the positive correlation was statistically significant only for aluminum.

Table 4 : Correlation coefficient between the levels of the measured parameters and severity of autism

Parameter	CARS r
Mercury (ug/g cr)	0.056
Lead (ug/g cr)	0.141
Arsenic (ug/g cr)	0.098
Cadmium (ug/g cr)	0.122
Aluminum (ug/g cr)	0.371**

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

DISCUSSION

Genes and the environment interact to change children's behaviors and skills. Egypt ranked as the first most dangerously polluted cities in the world[16]. Autism is a complex multifactorial epidemic all over the world. There are many causes that all work together[17]. So by studying a large number of children all over world (especially Egypt) we will discover which particular environmental exposures might result in autism or developmental delay. Results from our study showed that the heavy metals (mercury, lead , arsenic , cadmium and aluminum) were higher among the group suffering from autism compared to their controls and highest among those with sever autism compared to moderate and mild autism according to CARS.. However the difference were not statistically significant except for that of aluminum (P<0.001). Also there was positive correlation between the measured heavy metals and severity autism. However the positive correlation was statistically significant only for aluminum. Aluminum is absorbed from aluminum cookware, aluminum foil, antacids, antiperspirants, baking powder, buffered aspirin, canned acidic foods, food additives, lipstick, medications and drugs (anti-diarrheal agents, hemorrhoid medications, vaginal douches), processed cheese, "softened" water, and tap water. Target tissues are bones, brain, kidneys, and stomach. Signs and symptoms include colic, dementia, esophagitis, gastroenteritis, kidney damage, and liver damage[18]. Aluminum can elevate blood and brain glutamate to levels known to cause neurodegeneration in the developing brain and abnormal connectivity[19]. Our study added further support to the results of Lucija Tomljenovic and Christopher A. Shaw (2011) which stated that: (i) children from countries with the highest ASD prevalence appear to have the highest exposure to Al from vaccines; (ii) the increase in exposure to Al adjuvants significantly correlates with the increase in

ASD prevalence in the United States observed over the last two decades (Pearson r=0.92, pb0.0001); and (iii) a significant correlation exists between the amounts of Al administered to preschool children and the current prevalence of ASD in seven Westerncountries, particularly at 3–4 months of age (Pearson r=0.89–0.94, p=0.0018–0.0248). The application of the Hill's criteria to these data indicates that the correlation between Al in vaccines and ASD may be causal[20].

In a very important study aluminium chloride (100 mg/kg) was administered daily for six weeks that significantly increased cognitive dysfunction in the Morris water maze and oxidative damage as indicated by a rise in lipid peroxidation and nitrite concentration and depleted reduced glutathione, superoxide dismutase, catalase and glutathione S-transferase activity compared to sham treatment. Chronic aluminium chloride treatment also significantly increased acetylcholinesterase activity and the aluminium concentration in brain compared to sham (placebo treatment). Chronic administration of carvedilol (adrenergic antagonist) (2.5 and 5 mg/kg) daily to rats for a period of 6 weeks significantly improved the memory performance tasks of rats in the Morris water maze test, attenuated oxidative stress (reduced lipid peroxidation, nitrite concentration and restored reduced glutathione, superoxide dismutase, catalase and glutathione S-transferase activity), decreased acetylcholinesterase activity and aluminium concentration in aluminum treated rats compared to control rats ($p < 0.05$). Results of this study demonstrated the neuroprotective potential of carvedilol in aluminum chloride-induced cognitive dysfunction and oxidative damage[21].

Our results were in agreement with J. B. Adams et al (2009). This study demonstrates a significant positive association between the severity of autism and the relative body burden of toxic metals. This study investigated the relationship of children's autism symptoms with their toxic metal body burden and red blood cell (RBC) glutathione levels. Toxic metal body burden was assessed by measuring urinary excretion of toxic metals, both before and after oral dimercaptosuccinic acid (DMSA). Multiple positive correlations were found between the severity of autism and the urinary excretion of toxic metals[22].

Bernard et al. (2005)[23] discussed the many similarities between the symptoms of children with autism and children poisoned by mercury. An epidemiology study by Windham et al. (2002a)[24] found that the amount of airborne pollutants, and especially mercury, correlated with an increased risk for autism. A study by DeSoto and Hitlan, 2007[25] found that blood levels of mercury did significantly correlate with the diagnosis of autism. A small study by Adams et al., 2007[26] found that children with autism had a 2-time higher level of mercury in their baby teeth than typical children. A study by Bradstreet et al., 2003[27] investigated the body burden of toxic metals by giving dimercaptosuccinic acid (DMSA), an oral chelation medication approved by the FDA for treating infantile lead poisoning. They found that the children with autism excreted 3.1 times as much mercury into their urine (which is where DMSA is excreted), , but lead and cadmium levels were not significantly different. Overall there is some evidence to suggest that mercury and possibly other toxic metals are related to the etiology of autism.

The same results were also obtained by Geier et al., 2009[28] who found that elevations in urinary porphyrins (associated with mercury or lead and mercury toxicity) were significantly associated with Childhood Autism Rating (CARS) scores. This is the only study which used CARS as an assessment score.

A S Holmes et al, 2003[29] reported a relationship of the severity of autism with a biomarker which found a strong inverse relationship of the severity of autism with the amount of mercury in the baby hair of the subjects.

On the contrary, a replication study (J B Adams, 2004)[30] did not reproduce that correlation with severity. So, while two studies (A S Holmes et al, 2003 and Geier et al., 2009) do support a possible relationship of variations in the severity of autism with body burden of toxic metals, as was found in this paper.

Also Vergani Lauraet al.(2011)[31] investigated blood samples of autistic children and healthy controls and the plasma concentration of 13 metals (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Si, Zn) was measured by ICP-AES; Autistic children showed significantly higher plasma levels of Zn, Ca, Fe, As, Ni, Cd and Si. This study opposed our results because aluminium was not high in autistic children. Additional researchs is needed to confirm all findings.

CONCLUSION

Our results suggest a potential association between autism and the estimated metal concentrations, and possibly alluminium requiring confirmation and more refined exposure assessment in future studies.

Overall, the correlation analysis found multiple significant correlations of severity of autism and the urinary excretion of toxic metals, such that a higher body burden of toxic metals was associated with more severe autistic symptoms. However, the finding of a relationship does not establish causality.

Recommendation

Our suggetion opens the door to explore the hidden face of this annoying disease and we recommend to help in treatment by the following:

-Early screening of alluminium in urine in suspected and high risk children.

-Minimise environmental exposure as possible

-Chemical chelation of neurotoxic metals

-Decrease absorption by adding nutritional fibers as psyllium husk and bran to food.

-Antioxidants and vitamins to decrease brain inflammation.

-We recommend clinical trial with cardivilol (adrenergic antagonist).It may be a new treatment for autism in the future**.**

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