

# Impact of Some Toxic Factors Influencing Autism: A Case Control Study among Some Egyptian Children

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**Abstract** Autism is a neurodevelopmental disorder diagnosed clinically on the basis of deficits in social interaction, communication, and range of interests and activities. While, the neurobiologic basis for autism is now established, the specific etiology is unknown. The study tried to put hand on the most offending toxic risk factors for autism through detecting the levels of mercury and lead in the hair of autistic children and whether their levels are correlated with the changes in hair copper and zinc levels. Results showed the lead and mercury were detected in the hair samples of autistic children. The lead levels were significantly increased while mercury levels were significantly decreased in comparison to control group. There were changes in both copper and zinc levels in the hair samples of autistic children as copper levels were significantly higher in autistic cases than in control while, zinc levels were significantly lower in autistic cases than in control. There was positive correlation of Child Autism Rating Scale (CARS) and mercury levels while; Intelligence Quotient (IQ) has significant negative correlation with hair lead levels. By using logistic regression model in the current study, it is found that levels of hair mercury followed by copper then lead were associated with statistically significant increased risk of autism. In conclusion, infantile zinc deficiency and toxic metal accumulation may play principal roles in the pathogenesis of autism.

**Keywords** Toxic Autism, Heavy Metals, Mercury; Lead; Zinc; Copper

## Introduction

Autism is listed in the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition Text Revision (DSM IV-TR) as a mental disorder. Autistic disorder is correctly categorized as a complex neurobiological disorder that involves structural brain anomalies, deficits in neural connectivity, and a lack of integration and coherence in brain function. These impairments manifest in pervasive cognitive, social-emotional, and behavioral disturbances (American Psychiatric Association, 2000; Maser and Patterson, 2002).

The prevalence of autism currently reported in the US is 6–7 cases per 1000 children. This reported prevalence is substantially higher than that of a decade earlier. Similar increases have been noted in the United Kingdom, Europe and Japan. The centers for disease and prevention (CDC) survey that established the current United State rate found no significant difference between Caucasian and African-American children. It confirmed previous reports that autism

spectrum disorders (ASDs) is 3–5 times more common in boys (Rutter, 2005; CDC, 2007).

The link between exposure to heavy metal toxins and neurological brain damage associated with learning and behavioral disorders in children was concerned. It was shown that exposure to heavy metals such as lead and mercury can impair brain development at very early ages-even at low doses previously deemed "harmless". Children are particularly susceptible to the deleterious effects of heavy metal exposure for several reasons. First, their developing nervous systems are more sensitive than adults. Second, their bodies absorb toxins more rapidly, yet clear them from the system more slowly, than adults. Finally, a child's blood-brain barrier, the natural protective mechanism which blocks harmful substances from entering and damaging the brain, is not yet fully developed (Faber et al., 2009).

Adams et al., (2009) summarized the possible sources of heavy metals poisoning as chemical

products, fertilizers, industrial paint, building materials, fish that is high in mercury, silver dental fillings, and mercury preservatives in vaccines, nasal sprays, and many more. The importance of zinc in human nutrition and health has been recognized since the early 1960s. For assessment of zinc nutritional status, the use of serum zinc alone is subject to limitations because its level seems to be influenced by factors other than dietary zinc intake (Prasad, 2009).

The study tried to put hand on the most offending toxic risk factors for autism through detecting the levels of mercury and lead in the hair of autistic children and whether their levels are correlated with the changes in hair copper and zinc levels.

## Material and Methods

### Patients

Written informed consent was obtained from parents of all children involved in the present study. Children of the test group (n=32) were selected randomly from the phonetics and the psychiatric out-patient clinics along a period of nine months. All met DSM-IV-TR criteria (American Psychiatric Association, 1994) for the diagnosis of childhood autism. Children with other axis I psychiatric disorders were excluded.

Also children of smoker's mothers were excluded. All children had no history of chronic medical co morbid condition. The control group (n=32) included children who were matched regarding age and sex with the patients, without psychiatric or medical disorders.

All participating children (test and control groups) had received their full scheduled childhood immunization appropriate for their respective ages.

## Methods

### I- Psychiatric assessment

All the subjects were tested for:

- 1) The presence of psychiatric disorders and diagnosis of pervasive developmental disorder using the structured clinical Interview for DSM-IV Childhood Diagnosis (KID-SCID) (Matzner, 1994; Matzner et al., 1997).
- 2) The severity of autistic symptoms as measured by the Childhood Autism Rating Scale (CARS) (Schopler et al., 1986; Ozonoff et al., 2005). It consists of 15 categories, each rated on a 4 point scale. The individual is considered non- autistic when his total score fall in the range of 15-29, mildly-moderately autistic when his total score fall in the range of 30-36, and severely autistic from 37-60.
- 3) Intelligence quotient (IQ) (Binet and Simon, 1916).

### II. Samples collection

Hair specimens were obtained by accepted collection protocol, and standards established by Trace Elements, Inc., USA. Approximately 100 mg of scalp hair

samples were cut from each child with stainless-steel scissors from four to five different locations from the posterior vertex and posterior temporal regions of the scalp, as close to the scalp as possible, not exceeding 40 mm in length from the scalp. In the trace element laboratory, the hair specimen was cut into hair strands less than 3 mm in length and mixed to allow a representative sub sampling. After cutting, each specimen was weighed ( $\pm 1\text{mg}$ ) then washed consecutively in neutral detergent (ether) and dried before analysis according to the procedure originally described by Sorenson et al., (1973) and Vanloon (1985).

### III. Biochemical studies

Analysis of the studied heavy metals and trace elements [lead (Pb); mercury (Hg); copper (Cu) and zinc (Zn)] was done by Perkin Elmer 2380 Atomic Absorption Spectrophotometer after wet ashing of hair specimens using reagent-grade  $\text{HNO}_3$  and  $\text{HClO}_4$  according to Eads and Lambdin (1973) and Stockwell and Corns (1993). Instrument start-up and optimization were carried out as detailed in the operating manual. The source of the flame was an air-acetylene mixture. Hydride generation method was used for Hg. Wavelengths were set at 217, 253.7, 324.8 and 213.9 nm for Pb, Hg, Cu and Zn respectively.

## Results

Test and control groups were matched regarding the age as the mean  $\pm$  SD was  $4.1 \pm 0.8$  and  $4.03 \pm 0.8$  years respectively, and in both groups the ratio between boys and girls was 3:1. According to CARS, about 37.5% of the test group was classified as mild to moderate and 62.5% classified as severe autistic disorders. Regarding IQ, 87.5% were mild to severe mental retardation and 12.5% were below average mentality (Table 1 & 2).

Lead levels were significantly higher in autistic group than in control group ( $9.75 \pm 1.8$  and  $6.8 \pm 0.86 \mu\text{g}/\text{mg}$  respectively) while, mercury levels were significantly lower in autistic group than in control group ( $0.55 \pm 0.06$  and  $3.2 \pm 0.2 \mu\text{g}/\text{mg}$  respectively) as shown in table 3.

Copper levels were significantly higher in autistic group than in control group ( $26.5 \pm 1.9$  and  $19.1 \pm 4.4 \mu\text{g}/\text{mg}$  respectively) while, zinc levels were significantly lower in autistic group than in control group ( $304.99 \pm 25.8$  and  $419.5 \pm 45.96 \mu\text{g}/\text{mg}$  respectively) as shown in table 3.

CARS has significant positive correlation with both mercury ( $r = 0.615$  and  $P = 0.000$ ) and copper ( $r = 0.404$  and  $P = 0.010$ ) hair levels. While IQ has significant negative correlation with hair lead levels ( $r = -0.353$  and  $P = 0.026$ ). Hair zinc levels did not correlate with either CARS ( $r = 0.057$  and  $P = 0.727$ ) or IQ ( $r = 0.052$  and  $P = 0.750$ ) (Table 4).

By using logistic regression model, hair levels of mercury (0.021) then copper (0.026) then lead (0.041) were associated with statistically significant increased risk of autism (Table 5).

**Table (1): Classification of autistic group according to CARS.**

CARS	Autistic group (n=32)	Percentage (100%)
Non autistic (15-29)	0	0%
Mild to Moderate (30-36)	12	37.5%
Severe (37-60)	20	62.5%
Total	32	100%

*n*: number of cases.

**Table (2): Degree of IQ in autistic group.**

IQ	Autistic group (n=32)	Percentage (100%)
Mild to severe mental retardation (20-70)	28	87.5%
Below average mentality (71-89)	4	12.5%
Normal mentality (90- 109)	0	0%
Total	32	100%

*n*: number of cases.

**Table (3): Statistical comparison of hair lead, mercury, copper and zinc levels in the studied groups.**

Metals level (µg/mg)	Autistic group (n=32)	Control group (n=32)	T	<i>p</i>
	mean ± SD	mean ± SD		
Lead	9.75 ± 1.8	6.8 ± 0.86	8.624	<0.001*
Mercury	0.55 ± 0.06	3.2 ± 0.2	69.202	<0.001*
Copper	26.5 ± 1.9	19.1 ± 4.4	8.493	<0.001*
Zinc	304.99 ± 25.8	419.5 ± 45.96	11.358	<0.001*

\*Significant if  $P < 0.05$

If *p* is significant, it means there is a difference with statistical significance between both groups.

*n*: number of cases.

SD: standard deviation.

**Table (4): Correlation of the metals' level, CARS and IQ in the studied groups.**

Metals level (µg/mg)	CARS		IQ	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Lead	-0.175	0.281	-0.353	<0.026*
Mercury	0.615	<0.001*	-0.196	0.225
Copper	0.404	<0.010*	0.002	0.990
Zinc	0.057	0.727	0.052	0.750

\* Significant if  $P < 0.05$

If *p* is significant, it means the variable is highly correlated with either CARS or IQ.

*r*: correlation coefficient.

**Table (5): Risk factors of autism by using logistic regression model.**

Metals level (µg/mg)	B	SEE	<i>p</i>
Lead	-1.019	.498	<0.041*
Mercury	58.757	25.464	<0.021*
Copper	1.323	.596	<0.026*
Constant	-55.080	24.396	

\* Significant if  $P < 0.05$

*B*: regression coefficient.

SEE: standard error of estimates.

*P* Significant: it means the variable has a strong risk effect on the disease.

## Discussion

There is increasing suspicion that autism doesn't have a single cause but it is a complex disorder with a triad of (social impairment, repetitive behavior and communication difficulties) that have distinct causes but often co-occur (Freitag, 2007).

The current study tried to put hand on the most affecting toxic risk factor for autism through detecting the level of mercury and lead in the hair of autistic children and whether their levels are correlated with the changes in copper and zinc levels.

Regarding to CARS, 37.5% of the autistic group were classified as mild to moderate and 62.5% classified as severe autistic disorders. Regarding IQ, 87.5% were mild to severe mental retardation and 12.5% were below average mentality. Accordingly, El-Baz et al., (2011) in their study done in Cairo, 57% of their cases had severe degree of autism, 28% had moderate degree, and 15% had mild degree. In addition, 55% of their patients presented with mild to severe mental retardation, 36% with below average

mentality and 9% with normal mentality. Also, Baron-Cohen et al., (2006) reported that autistic children have spectrum of IQ ranged from 0 to 60 in a study in Autism Research Centre in Cambridge University.

The result of the present study showed significantly decreased level of mercury in the hair of autism children than the control group.

Mercury toxicity was first hypothesized as an etiology for autism in an article by Bernard et al., (2001), who noted the similarity of presentation between individuals with autism and those with mercury poisoning.

Williams et al., (2008) stated that measuring of chronic mercury exposure have also been problematic. Blood and urine have been used to ascertain acute exposure but are not as helpful in chronic exposure. Hair analysis has long been used as a measure of chronic exposure and is supported by the Agency for Toxic Substances and Disease Registry as a measure of methyl-mercury exposure (United States Department of Health & Human Services, 1999).

A study by Ip et al., (2004) looked at blood and hair samples of 82 children with autism compared to 55 typically developing peers and found no significant differences in mercury levels of both groups. Indeed, a report by Holmes et al., (2003) looking at first hair samples of 94 children with autism compared with 45 controls yielded decreased levels of mercury in children with autism in a study done in US.

In addition, Williams et al., (2008) in their study comparing children with autism to typically developing siblings found no clinically significant difference in mercury levels, although there was a slight trend toward lower levels in children with autism.

Geier et al., (2008) concluded that emerging evidence supports the theory that some autism spectrum disorders may result from a combination of genetic biochemical susceptibility, specifically reduced ability to excrete mercury and exposure to mercury at critical developmental periods. They also pointed the role played by protective factors (e.g., estrogen, glutathione, selenium and vitamins) and exacerbating factors (e.g., antibiotics, concurrent heavy metal exposure e.g., lead, arsenic and androgens).

This unexpected decreased level of mercury in the hair of autism children than in the control group in the current results can be explained with the assumption that children with autism are less able to metabolize and excrete mercury. Also may the current study is limited by the small number of participants; the results call into question the validity of hair analysis as means of measuring mercury exposure in patients with autism. In addition, findings threw a light on theories which propose mercury as an etiologic agent in this complex developmental disorder.

In the present study, hair lead levels were significantly higher in autistic children than the control group. However, there is no significant correlation between lead level and CARS (severity of autism) meanwhile; it correlated significantly negative with the IQ.

Lead is known to adversely promote inflammatory responses and or autoimmunity. Children at risk of autism may be particularly susceptible to chemical triggers that have biological targets shared by both the immune and the nervous systems (Pessah, 2006). This may be supported by Lanphear et al., (2005) who confirmed that low lead levels result in a drop of IQ and that a proportionally greater decrease was seen at lead levels < 7.5 µg/dl. This gives evidence for dangerous effect of lead poisoning on intelligence and may suggest different role of lead in the etiology of autism.

Results of the current study showed significantly increased copper levels in the hair of autistic children compared to the control group. While, it showed significantly decreased zinc levels in the hair of autistic children compared to the control group.

In accordance, Priya and Geetha (2011) have observed the children with different grades of autism to show highly significant level of copper in their hair and nail samples when compared to healthy controls. The level of Copper in the autistic children could be correlated with their degree of severity of autism.

According to Walsh (2001), metallothionein (MT) proteins are chains of amino acids present in cells of the brain, skin and GI tract. They assist in developing brain neurons, controlling copper and zinc levels, detoxifying heavy metals and supporting immune function. As consequences of MT dysfunction: Heavy metal Toxicity as MT is the body's primary protection against heavy metals, a magnet for mercury, lead, and cadmium. Intestinal MT prevents absorption of ingested toxic metals. Once bound to MT, toxic metals become inactive. Also in MT dysfunction, copper/zinc imbalances occur. Imbalanced Cu/Zn impairs the hippocampus and amygdala, which monitor social- emotional function. There is thus a tendency for emotional meltdowns, attentional deficits, and social isolation.

Zinc is an essential trace element that plays important role in nucleic acid/protein synthesis, cell replication, tissue growth and repair. Hair zinc level is commonly used in marginal zinc deficiency studies of children, and its usefulness has been documented in many industrialized countries including Canada and USA (Vaghri et al., 2011; Yasuda et al., 2011).

The mechanisms of zinc deficiency in autistic infants may be explained by their unbalanced nutritional intake and lower absorption ability in the intestinal duct (Adams et al., 2011).

In addition, maternal cigarette smoking has been reported to be associated with lower zinc and higher cadmium and lead concentrations in blood of their neonates (Razagui and Ghribi, 2005).

Whenever zinc becomes deficient, copper tends to accumulate (Yanik et al., 2004). The frequency of zinc deficiency, copper toxicity and low zinc/copper in children with autism spectrum disorders (ASDs) may indicate decrement in metallothionein system functioning. The plasma zinc/serum copper ratio may be a biomarker of heavy metal, particularly mercury, toxicity in children with ASDs (Faber et al., 2009).

Previous observations suggested that there may be an association between elevated serum and hair copper (Cu) levels and decreased serum and hair (Zn) levels and some psychiatric disorders. A relation between low concentrations of zinc and mental health problems, especially in at risk populations has been demonstrated. Zinc deficiency induced depression-like behavior in mice that was incompletely corrected by antidepressant therapy (Whittle et al., 2009).

From the results of the present study, it is evident that high levels of mercury and copper in hair are significantly associated with higher CARS. This was supported by Adams et al., (2009) who found that severity of a child's autism coincided with the levels of toxic metals excreted in their urine after treatment with metals removal therapy.

By using logistic regression model in the current study, it is found that levels of hair mercury followed by copper then lead were associated with statistically significant increased risk of autism.

## Conclusion

This study points to significant difference between autistic children and the control group regarding increased levels of lead and decreased levels of mercury in their hair. While, CARS correlated positively with mercury, the IQ correlated negatively with lead in their hair. By using logistic regression model, mercury then copper then lead hair levels was associated with statistically significant increased risk of autism. The present study showed that many of children with autistic spectrum disorders have been suffered from zinc deficiency and this finding suggested that zinc deficiency and toxic metal accumulation may play principal role in the pathogenesis of autism.

## Recommendation

It is important to reduce exposure to toxic metals as much as possible. Nutritional supplementation is recommended especially for children with autism who usually have a need for increased amounts of vitamins, minerals especially zinc, and some amino acids.

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## المخلص العربي مردود بعض العوامل السمية المؤثرة في التوحد: دراسة باستخدام مجموعة ضابطة في بعض الأطفال المصريين

سهيلة محمد الشربيني عطا الله و عفاف محمود عطية و سلوى طوبار<sup>1</sup> و إيمان الششتاوي<sup>2</sup>  
التوحد هو اضطراب النمو العصبي في الأطفال و يتم تشخيصه على أساس العجز المتفشي في التواصل الاجتماعي والتفاعل والأنشطة. و بالرغم من ذلك نجد أن مسببات التوحد المحددة ما زالت غير معروفة. تهدف الدراسة الحالية إلى إلقاء الضوء على أكثر المعادن السامة التي قد تؤثر كعامل خطر لمرض التوحد من خلال قياس مستويات الزئبق والرصاص في شعر الأطفال المصابين بالتوحد و عما إذا كانت ترتبط مستوياتها مع التغيرات التي طرأت على مستويات النحاس والزنك. وأظهرت النتائج وجود مادتي الرصاص والزنك في شعر الأطفال المصابين بالتوحد. وكانت مستويات الرصاص ذات دلالة إحصائية عالية في حين انخفضت بشكل ملحوظ مستويات الزئبق مقارنة بالمجموعة الضابطة. وكانت هناك تغييرات في كل من مستويات النحاس والزنك في شعر الأطفال المصابين بالتوحد حيث كانت مستويات النحاس أعلى في حالات التوحد مقارنة بالمجموعة الضابطة، بينما كانت مستويات الزنك أقل في حالات التوحد مقارنة بالمجموعة الضابطة كما كان هناك ارتباط إيجابي في نتائج المقابلة الإكلينيكية المصممة لتشخيص الأمراض النفسية تبعاً للدليل الأمريكي الرابع ومستويات الزئبق في شعر الأطفال في حين كانت هناك علاقة سلبية بين معدل الذكاء ومستويات الرصاص في الشعر. وباستخدام نموذج الانحدار اللوجستي، وجد أن مستويات الزئبق ثم النحاس ثم الرصاص في الشعر تمثل إحصائياً عوامل خطيرة في حدوث مرض التوحد. وقد استخلص من النتائج أن نقص الزنك لدي الأطفال وتراكم المعادن السامة يمكن أن يلعبوا الأدوار الرئيسية في التسبب في مرض التوحد.

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