Study of some biomarkers in hair of children with autism

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Introduction
Autism is a severe developmental disorder, which involves social withdrawal, communication deficits, and stereotypic repetitive behavior. The possible etiologies that precipitate autism symptoms remain controversial in many cases, but both genetic and environmental factors have been implicated. Mercury has gained much attention for a considerable period of time before other exacerbating or protective factors were suggested. The aim of this study was to investigate the relationship between autism and the level of some metals (namely mercury, lead, and copper) or zinc as a counteracting antioxidant element.

Methods
The study recruited 32 autistic children and 32 normal controls and all of them were subjected to KID-SCID, Childhood Autism Rating Scale (CARS), Stanford Binet intelligence test, and biochemical analysis of hair samples for the level of mercury, copper, lead and zinc.

Results
There were highly significant differences between the level of these substances in the hair of children with autism compared with controls, positive correlation of CARS score with both mercury and copper, while intelligence quotient has significant negative correlation with the level of lead in the hair. The level of zinc does not correlate with either CARS score or intelligence quotient.

Conclusion
These preliminary results suggest a complementary role for the studied elements in the pathogenesis of autistic disorder, which should be considered in the management plane.

Keywords:
autism, heavy metals, intelligence, trace elements

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Introduction

Autistic disorder is characterized by qualitative deficits in reciprocal social interaction and communication skills with restricted patterns of behavior. The estimated prevalence of autism varies widely from 1 in 166 for autism spectrum disorder (ASD) to 1 in 750 for the narrowest diagnostic criteria [1]. The reported rates of autism have increased sharply in the United States and in the United Kingdom. One possible factor underlying its rising prevalence is exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy [2]. However, Volkmar [3] pointed that the role of environmental factors in autism, particularly mumps, measles, rubella vaccine, is still controversial and the effect of thimerosal (a mercury-containing compound) has not been supported consistently in large controlled studies. Many lines of evidence point to similarities between mercury poisoning and autism, including similarities in clinical picture [4]. Mercury poisoning and autism have nearly identical symptoms, such as self-injurious behavior, social withdrawal, lack of eye contact, lack of facial expression, hypersensitivity to noise and touch, and repetitive behaviors [5]. Common affection of certain areas in the brain e.g., amygdala, hippocampus, basal ganglia, cerebral cortex [6], shared abnormalities in neurotransmitters namely serotonin, dopamine and epinephrine [7,8].

Much concern has been raised over the link between exposure to heavy metal toxins and neurological brain damage associated with learning and behavioral disorders in children. Researches show that exposure to heavy metals, such as lead and mercury, can impair brain development at very early ages, even at low doses that were earlier 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. Second, their bodies absorb toxins more rapidly; yet, the toxins are cleared from the system more slowly than adults. Finally, a child's blood–brain barrier, the natural

protective mechanism that blocks harmful substances from entering and damaging the brain, is not yet fully formed [9].

Some possible sources of heavy metal poisoning include chemical products, fertilizers, industrial paint, building materials, fish that is high in mercury, silver dental fillings, mercury preservatives in vaccines, nasal sprays, and many more. Lead may be found in the dirt near roads and is commonly present in paint from older houses. Children with pica or who eat paint chips may develop toxic lead levels. Children with autism may be less able to detoxify toxic agents to which they are exposed to from the environment, and this inability may predispose children to suffer neural damage consistent with autistic behavioral traits [10].

Geier *et al.* [11] suggested that emerging evidence supports the theory that some ASDs 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, such as lead, arsenic, and androgens).

As a preliminary step in understanding the possible role of some metals in the etiology of autism in our culture, this study aimed at detecting the difference in the level of mercury, lead, copper, and zinc in the hair of children with autism versus normal controls and whether the levels of these substances are correlated with the severity of autism or not.

Materials and methods

This was a cross-sectional study and was carried out at the Mansoura University Hospital. Thirty-two children were recruited from the phonetic and the psychiatric outpatient clinics after taking consent from their parents to join the study. Cases included all children diagnosed as autistic disorder according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, and excluded all children with another axis I psychiatric disorder or having another chronic medical comorbid condition.

A control group was selected, which included 32 children who were age-matched and sex-matched without any psychiatric or medical disorders.

All children were tested for, (i) the presence of psychiatric disorders and diagnosis of pervasive developmental disorder using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Childhood Diagnoses [12]. (ii) The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS) [13] using the Arabic version that was translated by El-Dafrawi. It consists of 15 categories, each rated on a four-point scale. The individual is considered nonautistic when his total score falls in the range of 15–29, mild-to-moderately autistic when his total score falls in the range of 30–36, and severely autistic when his total score falls in the range of 37–60. (iii) Intelligence quotient (IQ) using Stanford Binet Intelligence test [14], arabic version by Abdelsalam and Melika, was used.

Hair sample collection

Approximately 100 mg of scalp hair samples were cut from each child, both patients and controls, with stainless-steel scissors in the nape or occipital regions, as close to the scalp as possible. Hair collection was carried out in a dust-free environment and hair treated with artificial color was excluded from the study. The distal ends of the hair were cut from the samples. Each hair sample was comminuted, washed consecutively in neutral detergent, such as ether and acetone, and dried before analysis according to the procedure originally described [15,16].

Biochemical studies

Analysis of the studied heavy metals and trace elements [lead (Pb); mercury (Hg); copper (Cu), and zinc (Zn)] was carried out in the Toxicology Department by a Perkin Elmer 2380 (Perkin-Elmer, Atomic Absorption Spectrophotometer, United States) after wet ashing using reagent-grades HNO_3 and $HClO_4$ [17,18]. 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 mercury. Wavelengths were set at 217, 253.7, 324.8, and 213.9 nm for Pb, Hg, Cu, and Zn, respectively. Working standard solutions were prepared by appropriate dilution of stock solutions. Preparation of standards and samples was carried out under clean conditions using deionized water. All chemicals and reagents that were used were of ultra pure reagent grade (BDH Laboratory Reagents Ltd., Poole, England). All the glasswares were washed three times with deionized water, and then soaked in 20% nitric acid overnight. After soaking, the glasswares were rinsed three times with deionized water and dried. Quality assurance was achieved by measuring blank test solutions.

Results

Patients and controls were matched with regard to the age, ranging from 3.8 to 4.6 years with an average of 4.1 years. The *t*-test was carried out which showed no significant difference in age of children in both the groups. Chi-square test was carried out which showed significant difference in sex in both the groups (male children more than female children) (Table 1).

Results showed highly significant differences between the studied cases and the controls. The level of lead was significantly higher in cases (9.75 ± 1.8 ug/mg) than in controls (6.8 ± 0.86 μg/mg). The level of mercury was significantly lower in cases (0.55 ± 0.06 μg/mg) than in controls (3.2 ± 0.2 μg/mg). The level of copper was significantly higher in cases (26.5 ± 1.9 μg/mg) than in controls (19.1 ± 4.4 μg/mg). The level of zinc was significantly lower in cases (304.99 ± 25.8 μg/mg) than in controls (419.5 ± 45.96 μg/mg) (Table 2).

Table 1 Age and sex of the studied children

	Patients ($n=32$)	Control ($n=32$)	Statistical comparison
Age	4.1 ± 0.8 years	4 ± 0.8 years	$t=0.682$ $P=0.5$
Sex			
Male	24	24	$\chi^2=16$**
Female	8	8	$P=0.001$

**Highly significant if $P<0.01$.

Correlation studies were carried out, which showed that CARS score was significantly positively correlated with levels of both mercury ($r = 0.615$, $P = 0.000$) and copper ($r = 0.404$, $P = 0.022$) in the hair but insignificantly correlated with levels of lead ($r = -0.307$, $P = 0.087$) in the hair, whereas IQ was only significantly negatively correlated with levels of lead in the hair ($r = -0.402$, $P = 0.023$). The level of zinc does not correlate with either CARS score ($r = 0.102$, $P = 0.580$) or IQ ($r = 0.085$, $P = 0.645$) (Table 3). As shown in Table 4, a significant correlation between CARS score and sex (female) was found.

Discussion

The result of this study shows significantly decreased level of mercury in the hair of children with autism than controls. This was supported by Holmes *et al.* [2], who found low levels of mercury in the hair of children with autism, whereas another study carried out by Adams *et al.* [19] found normal levels. On interpreting their results, they stated that the normal level of mercury could indicate no abnormality in metal excretion, or they could indicate a combination of high metal body burden and low glutathione, yielding an average excretion rate of toxic metal into hair. In addition, current levels of mercury in hair are not a good way to check for mercury exposure during fetal development or infancy. However, lower levels of mercury in the hair of children with autism in this study and the positive correlation between mercury level and CARS scores may favor the lowered excretion explanation with higher body burden that can be supported by the negative correlation between the level of mercury in the hair and IQ.

The level of lead in hair is significantly higher in children with autism than controls. This result was the same as that of Fido *et al.* [20]. Lead is known to adversely promote inflammatory responses and/or autoimmunity.

Table 2 Comparison among levels of lead, mercury, copper and zinc in hair (microgram/milligram) in studied cases and controls

	Patients ($n=32$)	Control ($n=32$)	t	P
Lead (mean ± SD)	9.75 ± 1.8	6.8 ± 0.86	8.624**	0.000
Mercury (mean ± SD)	0.55 ± 0.06	3.2 ± 0.2	−69.202**	0.000
Copper (mean ± SD)	26.5 ± 1.9	19.1 ± 4.4	8.493**	0.000
Zinc (mean ± SD)	304.99 ± 25.8	419.5 ± 45.96	−11.358**	0.000

**Highly significant if $P<0.01$.

Table 3 Correlation of the level of substances in hair with Childhood Autism Rating Scale score and intelligence quotient in studied cases

	CARS score		IQ	
	R	P	R	P
Lead	−0.307	0.087	−0.402*	0.023
Mercury	0.615**	0.000	−0.090	0.626
Copper	0.404*	0.022	0.045	0.806
Zinc	0.102	0.580	0.085	0.645

CARS, Childhood Autism Rating Scale; IQ, intelligence quotient.
*Significant if $P<0.05$.
**Highly significant if $P<0.01$.

Children at risk of autism may be particularly susceptible to chemical triggers that have biological targets shared by both the immune and nervous systems [1].

However, there is no significant correlation between the level of lead and CARS scores (severity of autism). Meanwhile, it correlated significantly negatively with IQ. This was supported by Lanphear *et al.* [21] who confirmed that high levels of lead result in a drop of IQ and that a proportionally greater decrease is seen at levels of lead higher than 7.5 µg/dl. This gives evidence for dangerous effects of lead poisoning on intelligence and may suggest different roles of mercury and lead in the etiology of autism.

The study results show significantly increased levels of copper and decreased levels of zinc in the hair of children with autism compared with controls. Copper is a component of several metalloenzymes that are linked to dopamine synthesis, in biochemical pathways involving either antagonism of dopamine production or catalysis of its breakdown [22]. As dopamine is implicated in autism, copper homeostasis may be particularly relevant; an excess of copper may be associated with dopamine dysregulation. Zinc is required for structural integrity and/or catalysis of more than 200 enzymes, the majority of which are zinc metalloenzymes involved in nucleic acid and protein syntheses [23]. Whenever zinc becomes deficient, copper tends to accumulate [24]. Zinc and copper normally exist in a delicate balance. Zinc is a primary copper antagonist. When zinc is deficient, copper tends to accumulate in various storage organs.

The frequency of zinc deficiency, copper toxicity, and low zinc/copper in children with ASDs may indicate a decrease in the functioning of the metallothionein system. A retrospective review of plasma zinc, serum copper, and zinc/copper was done on data from 230 children with autism, pervasive developmental disorder not otherwise specified, and Asperger's syndrome. The entire cohort's

Table 4 Correlation between sex, Childhood Autism Rating Scale score, and intelligence quotient in the studied cases

	R	P
CARS score	−0.604**	0.000
IQ	−0.146	0.426

CARS, Childhood Autism Rating Scale; IQ, intelligence quotient.
**Highly significant if $P<0.01$.

mean zinc level was 77.2 μg/dl, mean copper level was 131.5 μg/dl, and mean Zn/Cu level was 0.608 μg/dl, which was below the 0.7 cutoff of the lowest 2.5% of healthy children. The plasma zinc/serum copper ratio may be a biomarker of heavy metal toxicity, particularly mercury toxicity, in children with ASDs [9].

In this study, it is evident that levels of mercury and copper in hair are significantly associated with higher CARS scores. This was supported by Adams *et al.* [19] who found that severity of a child's autism coincided with the levels of toxic metals excreted in their urine after treatment with metal removal therapy; the higher the levels of antimony and other metals excreted, the more severe was the child's autism.

Results also showed that the severity of autism is highly significant in female children with autism, which could be explained by the fact that, because autism is rarer in female children, it may take more number of risk genes for a female child to have autism. In addition, there is a possibility that there might be a biological difference in autism for female children versus male children [25].

The relatively low prevalence of the disorder and the economic cost limited the participant number and testing of other substrates in this study. However, the investigators tried to choose representatives of the protective and exacerbating factors that were suggested earlier.

Conclusion

This study points out significant differences between children with autism and controls with increased levels of lead and copper, and decreased levels of mercury and zinc in hair sample analysis. CARS scores correlated positively with mercury and copper, whereas the IQ correlated negatively with lead. These results suggest a complementary role for the studied elements in the pathogenesis of autistic disorder, which should be considered in the management plane.

It is important to first reduce exposure to toxic metals as much as possible and recommend nutritional supplementation especially for children with autism who have a need for increased amounts of vitamins, minerals, and some amino acids. More in-depth studies involving larger sample size to investigate the precise way of gene-environment interaction are recommended. In addition, population-based human epidemiological studies are needed to specify and explore the relationships between environmental chemical insults and the expression of key genes that are already linked to autism.

There is no conflict of interest to declare.

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الملخص العربي

دراسة بعض المؤشرات البيولوجية فى شعر الأطفال المصابون بالتوحد

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التوحد هو اضطراب نمائي شديد يتضمن انعزال اجتماعي و نقص في التواصل و تكرار نمطي للسلوك. و ما زالت العوامل المسببة لهذا الاضطراب غامضة و غير متفق عليها في كثير من الحالات و لكن تشير الدلائل إلي وجود عوامل متصلة بالوراثة وأخرى بيئية.

وقد حظت بعض تلك العوامل بمزيد من الاهتمام ومن أبرزها الزئبق بينما أشارت الدراسات الأحدث إلي اشتراك عوامل أخرى مرسبة او واقية. ولذلك كان الهدف من دراستنا الحالية هو تقصى مدى الارتباط والدور المحتمل لبعض المعادن في هذا الاضطراب,وقد تضمنت الدراسة الحالية 32 طفلا توحديا و 32 طفلا طبيعيا كعينة ضابطة وقد أجريت لهم المقابلة الإكلينيكية المصممة لتشخيص الامراض النفسية تبعا للدليل الاحصائى الامريكى الرابع. وقد أظهرت النتائج فروق ذات دلالة إحصائية فى مستوى كل من الزئبق والرصاص والنحاس والزنك فى شعر الأطفال التو حديين عنه فى العينة الضابطة. كما أظهرت علاقة ارتباطيه موجبة بين كل من الزئبق والنحاس, بينما ظهرت علاقة عكسية بين مستوى الرصاص ومعامل الذكاء, وفى الوقت نفسه لم تظهر اى علاقة بين مستوى الزنك وشدة التوحد أو معامل الذكاء.

وتشير نتائج هذه الدراسة المبدئية إلى وجود دور تكاملي للمعادن المذكورة فى إحداث أو ظهور أعراض التوحد مما يجب وضعه فى الاعتبار عند وضع خطة العلاج بعد التأكد منه بإجراء المزيد من الأبحاث على عدد اكبر من الحالات وتقصى مستوى عدد اكبر من العوامل فى عينات من الشعر والدم معا.