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
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 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 HNO₃ and HClO₄ [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 µg/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).
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>
<thead>
<tr>
<th>Table 2 Comparison among levels of lead, mercury, copper and zinc in hair (microgram/milligram) in studied cases and controls</th>
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<tbody>
<tr>
<td><strong>Patients</strong> $(n=32)$</td>
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<tr>
<td>Lead (mean ± SD)</td>
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<tr>
<td>Mercury (mean ± SD)</td>
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<tr>
<td>Copper (mean ± SD)</td>
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<td>Zinc (mean ± SD)</td>
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**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
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 CARDs 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. CARDS 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.

References

المملصع العربي
دراسة بعض المؤشرات البيولوجية في شعر الأطفال المصابون بالتوحد

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التوحد هو الاضطراب النفسي الذي يتضمن انزعاج اجتماعي و تقسيم في التواصل و تكرار نمط السلوكي، وما زالت العوامل المسببة لهذا الاضطراب غامضة و غير معقولة فيها في كثير من الحالات، ولكن تتميز باقل الانتهاء إلى وجود عوامل متصلة بالوراثة أو أخرى بيئية.

وقد جملت بعض تلك العوامل مزيد من الاهتمام من أبرزها الزنبق بينما أشارت الدراسات الأحدث إلى اشتراك عوامل أخرى مرتبطة أو واقية. وكذلك كان الهدف من دراستنا الحالية هو تقسيم مدى الارتباط والدور المحتمل لبعض المعاند في هذا الاضطراب. و قد تضمنت الدراسة الحالية 32 طفلاً و 32 فللا طبيعياً كعينة مشابهة، وقد أجري لها المقابلة الإكلينيكية المصممة لتشخيص الأمراض النفسية تبعاً للدليل الإحصائي الأمريكي الرابع. وقد أظهرت النتائج فروق ذات دلالة إحصائية في مستوى كل من الزنبق والرقصاد والنحاس والزنك في شعر الأطفال ذو احتقين عنه في العينة الضابطة. كما أظهرت علاقة ارتباطات موجبة بين كل من الزنبق والرقصاد، بينما ظهرت علاقة عكسية بين مستوى الرقصاد ومعامل الذكاء، وفي الوقت نفسه لم تظهر أي علاقة بين مستوى الزنك وشدة التوحد أو معامل الذكاء.

وتشير نتائج هذه الدراسة الميدانية إلى وجود دور تكميلي للمعادن المذكورة في إحداث أو ظهور أعراض التوحد مما يجب وضعيه في الاعتبار عند وضع خطة العلاج بعد التأكد منه بإجراء المزيد من الأبحاث على عدد أكبر من الحالات وتقسيم مستوى عدد أكبر من العامل في عينات من الشعر والماء.