Heavy metals and trace elements in hair samples of autistic children in central Saudi Arabia

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ABSTRACT

Objectives: Autism a childhood neurodevelopmental disorder, with onset prior to 36 months old. The etiology of autism is complex, and usually, the underlying pathologic mechanisms are unknown. Recently, alteration in heavy metals and trace elements had some interest. The aim of the present study is to examine levels of trace elements and heavy metals in hair samples, of autism spectrum disorders in the Riyadh area.

Methods: The study was conducted in Riyadh area, Kingdom of Saudi Arabia between September 2003 and April 2004. Seventy-seven autistic children participated in the study, all with confirmed diagnosis according to E-2 diagnostic criteria for autistic spectrum disorders. Hair samples were analyzed by atomic absorption spectrophotometer. The measurements of 9 heavy metals (lead, mercury, aluminum, arsenic, barium, cadmium, nickel, antimony and strontium), and 11 trace elements (sodium, calcium, chromium, copper, iron, magnesium, manganese, zinc, cobalt, selenium and molybdenum) was carried out.

Results: The current study showed significantly higher levels of toxic heavy metals mercury, lead, arsenic, antimony and cadmium in autistic spectrum disorders as compared to the control children. Moreover, hair samples from children with autistic spectrum disorders contained significantly lower concentrations of calcium, copper, chromium, manganese, iron and cobalt, as compared to normal children. In addition, we found a significantly higher incidence of social withdrawal, sleeping and eating disorders, speech and language disorders among autism spectrum disorders as compared to controls.

Conclusions: The present study demonstrated alteration in levels of toxic heavy metals and essential trace elements in children with autistic spectrum disorders as compared to normal children. This suggests a possible pathophysiological role of heavy metals and trace elements in the genesis of symptoms of autism spectrum disorders, such as social withdrawal, eating and sleeping disorders. In turn, those children with autistic spectrum disorders might benefit from chelating therapy for heavy metal poisoning and supplementation of essential trace elements.

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Autism is a neurodevelopmental syndrome with an onset prior to 36 months. It is a syndrome characterized by impairment in social related communication, repetitive behavior, abnormal movements and sensory dysfunction. Other characteristics include, inappropriate laughing and giggling, little or no eye contact, apparent insensitivity to pain, preference to be alone and many more characteristics.[1] Recent epidemiological studies suggested that autism might affect one in 150 American children. In the last 20 years, there has been an increase in the diagnosis of autism, which we cannot explain by genetics alone nor can this increase be secondary to only increased awareness. The etiology of autism is complex, and usually, the underlying pathologic mechanisms are

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unknown. Recent research has investigated genetic as well as neurochemical and immunological factors. In addition, heavy metals had some interest.[1-4] The human brain forms and develops over a long period, compared to other organs, with neuron proliferation and migration continuing in the postnatal period. The blood brain barrier is not fully developed until the middle of the first year of life. Furthermore, there is postnatal activity in the development of neuronal receptors and transmitter system, as well as the production of myelin. The fetus receives significant exposure to toxic substances through maternal blood and across the placenta, with the fetal level of toxic metals often being higher than that of maternal blood.[5-8] The incidence of immune reactive conditions such as autism, schizophrenia, attention deficit disorder (ADD), dyslexia, and learning disabilities, have been increasing rapidly in recent years.[9] Exposure to toxic chemicals or environmental factors appears to be a factor in as much as 28% of the 4 million children born each year.[10] With one in 6 having one of the neurological conditions listed above, the USA government estimates that over 3 million of these are related to lead or mercury toxicity.[10] Since autism has become one of the disease that causes impairment in children of Saudi Arabia and its etiology still not clear, the aim of this study is to reveal any cross-link between neurodevelopmental delay of autistic spectrum disorders and exposure to high concentration of heavy metals or deficit of essential trace elements early in life. For this purpose, we carried out measurement of heavy metals and essential trace elements in hair samples of autistics.

Methods. The study was conducted in Riyadh, Kingdom of Saudi Arabia, between September 2003 and April 2004. Seventy-seven children, aged up to 14 years old, with confirmed professional diagnoses of one of the following disorders were selected: autism, ADD, Rett's syndrome and Aspergers syndrome. The diagnosis was carried out either by a qualified psychologist, psychiatrist or neurologist, according to diagnostic criteria E-2 (Rimland 1968).[11] Written consent was obtained from parents prior to the start of the study, and then the parents were asked to complete a questionnaire regarding the child's medical and behavioral history. Healthy age and sex matched control volunteers were recruited from King Khaled University Hospital. Hair samples were collected in biohazard bags; the hair was obtained from the nap area (approximately one gm in weight).

Measurement of trace elements. Measurement of heavy metals and trace elements was conducted according to the method by Ludwig et al,[12] as follows: Hair samples were first rinsed with acetone and soaked for 1-2 hours in distilled demonized water. Then mixed and washed 3 times for one hour in a solution containing 0.1% Triton Tx-100 (Sigma Chemical Co., St. Louis, MO). Each sample was digested using 2 ml of 70% nitric acid and one ml of 30% hydrogen peroxide. Then hair samples were placed in polyfluoroethylene vessels and acid digested with a microwave digestion system at controlled pressure (150 psi) and temperature (110°C). Then the samples were ready to be tested for heavy metals by atomic absorption spectrometry. The measurements of 9 heavy metals (lead, mercury, aluminum, arsenic, barium, cadmium, nickel, antimony and, strontium), and 11 trace elements (sodium, calcium, chromium, copper, iron, magnesium, manganese, zinc, cobalt, selenium and, molybdenum) were conducted by atomic absorption spectrophotometer (Central Laboratory, Center for Female Studies, Al-Malaz). The precision and accuracy of the method were studied according to reference material CRM 398. The results were analyzed using SPSS for windows. Results were expressed as mean ± SEM. Statistical analyses for differences among the groups were assessed by t-test. *P*-values < 0.05 were considered significant.

Results. Seventy-one males (92.2%) and 6 females (7.8%) with autistic spectrum disorders participated in the study, including 65 autistics with a confirmed diagnosis (61 males and 4 females), 8 males with ADD, 2 males with Aspergers syndrome, and 2 females with Rett's syndrome. The mean age for the total number of autistic children participating in the study was 8.8±0.5 years. The male to female ratio was 11.8:1. The percentages of children suffering from social withdrawal, sleeping and eating disorders and speech and language deficits in early childhood autism, ADD, Rett's syndrome and Aspergers syndrome are shown in **Table 1.** The concentration of essential trace elements and toxic heavy metals in hair samples of normal and autistic spectrum disorders is displayed in **Tables 2 & 3.** Hair samples from autistics and children with Aspergers syndrome contained significantly higher concentration of the toxic heavy metals mercury, lead, arsenic, antimony and cadmium. Whereas, hair samples from children with Rett's syndrome and ADD contained significantly higher concentration of lead. Furthermore, hair samples from autistics contained significantly lower concentrations of the following essential trace elements: calcium, copper, chromium, manganese, magnesium, iron, selenium and cobalt. Furthermore, hair samples from children with ADD contained significantly lower concentrations of calcium, copper, chromium, manganese, iron, molybdenum and cobalt. Moreover, hair samples from children with Aspergers and Rett's syndrome contained

Table 1 - Percentages of social withdrawal, sleeping, eating disorders and speech and language deficits in children with early childhood autism, attention deficit disorder (ADD), Rett's syndrome, and Aspergers syndrome regression.

Disorders	Total	Social withdrawal		Sleeping disorders		Eating disorders		Speech & language deficits	
		n	(%)	n	(%)	n	(%)	n	(%)
Control	80	10	(8)	14	(18)	14	(18)	4	(5)
Autism	65	55	(85)	31	(39)	50	(77)	54	(83)
Attention deficit disorder	8	7	(87.5)	3	(37.5)	7	(87.5)	7	(87.5)
Aspergers syndrome	2	2	(100)	1	(50)	1	(50)	2	(100)
Rett's syndrome	2	2	(100)	1	(50)	1	(50)	2	(100)

Table 2 - Concentration of heavy metals (ppm) in hair samples of children with autism, ADD, Aspergers syndrome and Rett's syndrome as compared to normal children.

Heavy metals	Normal	Autistic	ADD	Aspergers syndrome	Rett's syndrome
Mercury (Hg)	0.713±0.228	4.204±1.129**	0.51±0.028	1.941±0.046 *	0.89±0.09
Lead (Pb)	0.96±0.16	3.48±0.22**	4.2±0.16*	3.68±1.4*	1.4±0.8*
Arsenic (As)	0.23±0.05	1.8±0.16**	0*	2.86±0.09*	0*
Cadmium (Cd)	0.003±0.001	0.008±0.003*	0*	0.07±0.05*	0*
Barium (Ba)	0.006±0.02	0.001±0.009	0*	0*	0*
Antimony (Sb)	0.002±0.007	0.02±0.01*	0*	0*	0*
Aluminum (Al)	2.4±0.8	2.2±1.2	3.3±0.8	2±0.6	2.8±0.4
Strontium (Sr)	0.01±0.002	0.003±0.001	0.007±0.005	0.001±0.003	0.03±0.01
Nickel (Ni)	0.42±0.03	0.18±0.02	0.01±0.03*	0.06±0.008*	0.35±0.2
Age (year)	7.2±0.7	9±0.3	6±0.8	10.2±0.9	9±2

Values are mean ± SEM, * - Significant, ** - Highly significant, ADD - attention deficit disorder, ppm - parts per million

Table 3 - Concentration of trace elements (ppm) in hair samples of children with autism, ADD, Aspergers syndrome and Rett's syndrome as compared to normal children.

Trace elements	Normal	Autistic	ADD	Aspergers syndrome	Rett's syndrome
Zinc (Zn)	140±8	150±20	145±17	144±29	130±14
Sodium (Na)	5±0.4	4.2±0.3	4.2±0.3	4±0.1	4.2±0.3
Calcium (Ca)	830±20	480±30**	480±3**	390±3**	530±3**
Copper (Cu)	14±0.2	3±0.2**	3±0.2*	3±0.2*	3.7±0.2*
Chromium (Cr)	0.14±0.01	0.04±0.01**	0.03±0.01**	0.04±0.1*	0.01±0.01**
Manganese (Mn)	0.6±0.06	0.33±0.04*	0.33±0.04*	0.4±0.04	0.32±0.04*
Magnesium (Mg)	5±1	3±1**	3±1*	3.4±1	4.2±1
Iron (Fe)	5±0.5	2±0.2**	2±0.2**	2±0.2**	2.2±0.4**
Cobalt (Co)	0.2±0.02	0.1±0.01*	0.1±0.01	0.2±0.1	0.2±0.01
Selenium (Se)	0.9±0.04	1.2±0.05**	1.2±0.05*	1±0.05	0.9±0.03
Molybdenum (Mo)	0.03±0.004*	0.03±0.003	0.003±0.004*	0.003±0.003*	0*
Age (year)	7.2±0.7	9±0.3	6±0.8	10.2±0.9	9±2

Values are mean ± SEM, * - significant, ** - highly significant, ADD - attention deficit disorder, ppm - parts per million

significantly lower concentrations of the following essential trace elements: calcium, copper, chromium, manganese, molybdenum and iron.

Discussion. Seventy-seven children with autistic spectrum disorders participated in the study. Sixty-five are autistic, with 61 were males and 4 females. The male to female ratio was 15:1, more than the known international ratio of 4:1. This is probably resulting from more males participating in the study than females. Furthermore, the study also included 2 males with Aspergers syndrome, and 2 females with Rett's syndrome. The major findings of this study were that children with autistic spectrum disorders were characterized by significantly higher levels of some heavy metals (such as mercury, lead, arsenic, antimony and cadmium), and significantly lower levels of some essentials trace elements (such as calcium, copper, chromium, manganese and iron) in hair samples. Moreover, the autistic spectrum disorders were associated with social withdrawal, and a higher incidence of sleeping and eating disorders, and a higher incidence of speech and language disorders, as compared to the control children. Our findings are consistent with others.[4,13,14] They reported significant differences in trace elements and heavy metals concentration in hair samples from children with behavioral abnormalities.

Scientist have found that heavy metals such as mercury, cadmium, lead and aluminum affects chemical synaptic transmission in the brain and the peripheral and central nervous system (CNS).[15,16] Those elements can lead to disruption of brain and cellular calcium levels that significantly affect several functions, such as calcium dependent neurotransmitter release, which results in depressed level of serotonin, norepinephrine and acetylcholine.[17,18] Those neurotransmitters are related to mood and motivation. Toxic metals have also been found to affect cellular transfer and levels of other important minerals and nutrients that have significant neurological, and health effects such as magnesium, lithium, zinc, iron, and vitamin B6, B1, B12.[19,20] Arsenic, like most of the other metals, has been found to be associated with neurological disorders. Areas with a higher water arsenic level, are associated with higher fetal and infant mortality, developmental delay, diminished intellectual ability and attention deficit disorders, compared to areas with a lower water arsenic content.[21] High aluminum levels have been found to be related to encephalopathies and dementia.[22] Some studies suggested that aluminum contributes to neurological disorders such as Alzheimer's disease, Parkinson disease, senile and pre-senile dementia, clumsiness of movement and staggering while walking.[23] Trace elements such as zinc, copper, lead and manganese, all alter adenylate cyclase activity, this in turn affects neurotransmitter metabolism, and consequently produces neurochemical and neurophysiological changes.[15] An example is manganese, which produces a profound influence on dopamine metabolism.[24] Trace elements may also affect the metabolism of serotonin, gamma-amino butyric acid and neuropeptides.[25] In addition, electrophysiological studies have shown that many cations including zinc, nickel, cobalt and magnesium, decrease the excitability of the CNS.[25] In 1985, Wecker et al[4] examined the concentration of 14 elements in scalp hair samples from control, autistic and autistic-like children. Results showed significantly lower levels of calcium, magnesium, copper, manganese, and chromium. They concluded that concentration of trace elements in hair from normal children differed from patterns observed in both autistic and autistic-like children. Furthermore, they suggested that hair analysis might have a potential use as a diagnostic tool for autism.[4]

Neurochemical and neurophysiological evidence indicated that trace elements markedly affect the metabolism of neurotransmitters in the brain. Behavioral studies showed that alteration in the dietary intake of elements such as iron, manganese, copper or zinc could lead to seizures, analgesia, yawning-stretching syndrome and circulating behavior in rats.[26] Exposure to mercury can cause immune, sensory, neurological, motor and behavioral dysfunction similar to symptoms and signs associated with autism. Thimerosal, a preservative added to many vaccines, has become a major source of mercury in children in their first years; many have received a quantity of mercury that exceeds safety guidelines.[27] Since 1999, thimerosal, has been removed from most vaccines routinely recommended for infants and children, in Europe, America, and some countries in Asia including Saudi Arabia. However, it is still used in the injectable influenza vaccine. Some infants and toddlers may be exposed up to 35-125% over what is considered safe, through vaccination. Many parents of autistic children thought symptoms worsened after receiving a vaccine.[27] Some parents noticed that the timing of mercury administration via vaccine coincides with the onset of autistic syndromes.[28]

Heavy metals (including lead, mercury, cadmium, nickel, aluminum) tend to concentrate in air and the food chain along with other toxic metals, facilitating metal poisoning, which is the most widespread environmental disorder.[2,9] Mercury and cadmium from combustion emission is also accumulating in costal and inland areas.[29] Lead exposure is primarily from soil, paint chips, drinking water, fertilizers, food, ceramics, cosmetics, gasoline, newsprint and colored advertisement, rubbers toys, tap water, tobacco smoke, and dust.[10] High levels of cadmium are found in regions with high emissions from

incinerators and car exhausts, as well as in shellfish, art supplies, cigarette smoke, processed food, fertilizers, fresh water fish, and batteries. Common exposure to aluminum includes aluminum cookware, antiperspirant, processed cheese, processed food, medication (anti-diarrheal agents), softened water, and tap water. Whereas common sources of arsenic include antibiotics given to commercial livestock, air pollution, drinking water, fish, shellfish, herbicides, and meat from commercially raised poultry. Alternatively, nickel, which is highly toxic and causes an immune reaction, is commonly found in dental crowns and jewelry. Manganese and other metal exposure such as cadmium, mercury, arsenic, silver, and copper can occur through drinking water and dental materials.[30,31] The most common significant exposure for most people is to mercury vapor from amalgam filling.[8] Likewise, a major exposure source for infant and young children is from placental transfer from their mother's amalgam filling and breast feeding.[8]

Results from the current study demonstrated significantly higher levels of toxic heavy metals in hair samples from children with autistic spectrum disorders, such as, mercury, cadmium, lead, antimony and arsenic. The most likely explanation is as mentioned earlier, due to exposure to toxic metals through drinking water, combustion emission, soil, paint chips, fertilizers, food, car exhausts, and so forth. However, a question might be raised: children with autism spectrum disorders are not the only children exposed to those toxic hazardous conditions, normal children are also exposed. The possible explanation for this is that autistic children might lack the ability to detoxify toxins, resulting in an accumulation of toxic substances in the body, and leading to alterations in biochemical processes taking place in the body. Therefore, heavy metal chelation therapy is necessary in some cases. In contrast, children with autistic spectrum disorders displayed lower levels of some essential trace elements such as calcium, copper, chromium, manganese, magnesium, iron, selenium and cobalt. This might be due to less oral intake, as they are very meticulous eaters with eating problems. Daily oral supplementation should be considered to overcome this.

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Dedication

This work is dedicated to my colleague the late Dr. Ahmad Al-JarAllah (Consultant Neuropediatrician, King Khaled University Hospital, Riyadh), who gave me all the support I needed when conducting my research on Autism, but did not live to see it completed. "May Allah grant him Jannah (Amen)."

From the Editors: The Editors and Staff of Neurosciences extend their heartfelt condolences to the family, friends and colleagues of Dr. Ahmad Al-JarAllah, a distinguished and respected member of the Neurosciences Community.