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Heavy Metals and Trace Elements in Hair and Urine of a Sample of Arab Children with Autistic Spectrum Disorder

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ABSTRACT

General information: *Autism is a severe developmental disorder which involves social withdrawal, communication deficits, and stereotypic/repetitive behavior. The pathophysiological etiologies which precipitate autism symptoms remain elusive and controversial in many cases, but both genetic and environmental factors (and their interactions) have been implicated. While autism is considered multi-causal, environmental factors have received significant attention. International discussion has focused on neurotoxins such as mercury and lead, suggesting that these and other toxic metals contribute to the development of the disorder. An epidemiological study released in 2006 (Palmer et al.) linking Toxic Release Inventory (TRI) data on mercury to special education data in Texas reported a 61% increase in autism prevalence rates (or 17% adjusted) per 1000 pounds of mercury released into the environment (1). We attempted to further evaluate whether exposure to variable environmental contributes to the genesis of autistic spectrum disorder, and thus is a factor increasing the risk for developing autism symptoms in utero or in early childhood.*

Purpose: *The purpose of this study is to examine possible environmental risk factors and sources of exposure to mercury and other heavy metals in children with autism spectrum disorder versus controls. Through laboratory diagnostics we are able to distinguish between present and past exposure (i.e. hair analysis measurements reflect past exposure), urinary excretion levels of unprovoked urine represent immediate exposure. By assessing a spectrum of trace elements and heavy metals in hair and urine of both autistic and control groups, we focused on the participants' past and present exposure.*

Methodology: *The participants were 25 Autistic Spectrum Disorder (ASD) children (22 boys and 3 girls) between the age of 3 and 9 years. They were either diagnosed previously by other psychiatrist, psychologist, and developmental pediatrician or suspected by their parents as being autistic. All children were attendants to the Child Psychiatric Clinic in Erfan Psychiatric Hospital in Jeddah, KSA. Samples were collected during the period of June 2006 to March 2008. A control group of 25 children without any psychiatric or medical disorders was age-matched and sex-matched. All parents signed informed consent forms. All autistic children were subjected to a full clinical child psychiatric sheet for the diagnosis of autism spectrum disorder and exclusion of other psychiatric disorders according to the Diagnostic and*

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Statistical Manual of Mental Disorders, 4th Edition (DSM IV). The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) using the Arabic versions. Both groups were subjected to the Questionnaire on Exposure to Heavy Metals, Physical Symptoms, and Child Development. Hair and baseline urine samples (i.e. unprovoked urine) were taken from both groups and sent to the German clinical and environmental laboratory Micro Trace Minerals Gmbh, for the detection of heavy metals and trace elements levels where metal testing was performed via ICP-MS spectroscopy utilizing cell technique.

Results: *By comparing the ASD Group to the Control Group, we found a statistically significant difference in the mean hair levels of arsenic, cadmium, barium, cerium and lead (p=0.01, 0.03, 0.003, 0.003, and 0.03 respectively), and in the mean hair levels of magnesium and zinc (p=0.001 and 0.003 respectively). There were also statistically significant differences in the mean urine levels of aluminum, barium, cerium, mercury, and lead (p=0.004, 002, 0.014, 0.006 and 0.004 respectively), and in the mean urine levels of copper and germanium (p=0.049 and 0.02 respectively). An agreement was found in both specimen (hair and urine) for barium and lead. The statistically significant differences in mean hair levels of arsenic, cadmium, and cerium were not supported by urine baseline levels. Also, the statistically significant magnesium and zinc levels of hair were not supported by urine levels. A disagreement was also found with copper and germanium concentrations.*

INTRODUCTION

Autism is a severe developmental disorder which involves social withdrawal, communication deficits, and stereotypic/repetitive behavior. The pathophysiological etiologies which precipitate autism symptoms remain elusive and controversial in many cases, but both genetic and environmental factors (and their interactions) have been implicated. One environmental factor that has received significant attention is the body burden of mercury, lead, and other toxic metals (2-4). Overall there is some evidence to suggest that the neurotoxic metal mercury and possibly other toxic metals are related to the etiology of autism. An epidemiology study by Windham et al. (5) found that the children with autism excreted 3.1 times as much mercury into their urine, suggesting increased exposure. Lead and cadmium levels were not significantly different.

This points to the importance of environmental factors and raises the possibility of an etiological role for toxic exposures: either prenatal, postnatal, or in some cumulative pattern that combines the effect of maternal, gestational, and infant exposures (6). 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-containing preservatives (thiomersal) in vaccines, nasal sprays, and many more. Lead may be found in the dirt near roads and can still be found in paint from older houses. Children eating paint chips or those with pica may develop toxic lead levels.

Genetically, children with autism may be less able to detoxify toxic environmental agents, and this inability may predispose them to suffer neural damage consistent with autistic behavioral traits (7). Women with chronic metal exposure (who have accumulated high tissue levels of mercury and other metals) may pass potentially toxic metals on to the fetuses, or intoxicate infants through nursing (8). Several factors are known to significantly increase the toxicity of mercury. One important factor is the synergistic toxicity with other metals, and an inefficient excretion mechanism. In the presence of an inefficient detoxification system, multiple toxic exposures may result in toxicity symptoms, even if exposure happens at a comparatively low level for each single toxicant. Wecker et al. concluded that concentration of trace elements in hair from normal children differ from patterns observed in both autistic and autistic-like children (9). Heavy metals are considered as reproductive and developmental toxins, they can cause birth defect and fetal developmental damage, neurological defects, developmental delay, learning disabilities and behavioral abnormalities.

To detect chronic metal exposure, hair analysis may have a potential use as a diagnostic tool for autism (10). Blaurock et al. documented that hair and urine mineral analysis levels support each other (11). This is of interest, be-

cause hair analysis evaluates past exposure while urine analysis detects immediate exposure. As documented in the Punjab study by Blaurock-Busch et al., if immediate exposure – as diagnosed in urine- remains for a period of weeks, months or even longer periods, it contributes to, and in fact causes long term exposure as reflected in hair.

We hypothesized that exposure to variable environmental risk factors may affect tissue concentration of heavy metals and trace elements, thus contributing to the genesis of autistic spectrum disorder. This raises the possibility of an etiological role for toxic exposures: either prenatal, postnatal, or in some cumulative pattern that combines the effect of maternal, gestational and infant exposure. We aimed to examine possible environmental risk factors and sources of exposure to mercury and other heavy metals in children with autism spectrum disorder versus controls. We assessed the levels of trace elements and heavy metals in hair and urine in both autistic and control groups, in the hope of establishing links between environmental exposure to both heavy metals and trace elements contributing to the genesis of autistic spectrum disorder. ❑

SUBJECTS AND METHODS

The participants were 25 Autistic spectrum disorder (ASD) children (22 boys and 3girls) between the age of 3 and 9 years {23 were diagnosed as autistic, one child was diagnosed with Asperger Syndrome, and one with pervasive developmental disorder (NOS)}. The children were either diagnosed previously by other psychiatrists, psychologists, and developmental pediatricians or were suspected by their parents as being autistic. All children attended the child psychiatric clinic of the Erfan Psychiatric Hospital in Jeddah, KSA. Samples were collected during the period of June 2006 to March 2008. All parents signed informed consent forms. A control group was selected, which included 25 age-matched and sex-matched children without any psychiatric, medical disorders or developmental delay. These children were friends and neighbors, unrelated to the study group.

The following entry criteria were applied:

1. No mercury amalgam dental
2. No previous use of DMSA or other prescription chelators.
3. No anemia or current treatment for iron-deficiency anemia.
4. No known allergies to DMSA.
5. No liver or kidney disease.
6. Children are well hydrated, receiving adequate daily intake of water.

Exclusion criteria included refusal to participate, physically handicapped children and children with progressive neurological disorders and unstable epilepsy. We excluded children who were taking regular medications including stimulants, anticonvulsants, and atypical antipsychotic drugs.

All of the children admitted to the study received routine childhood vaccinations, none had received chelation therapy. All autistic children were subjected to a full clinical child psychiatric sheet for diagnosis of autism spectrum disorder and exclusion of other psychiatric disorder according to Diagnostic and Statistical Manual of Mental Disorders 4th Edition, (DSM IV).

Also, the severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS) (12), translated by El-Dafrawi. It consists of 15 categories, each rated on a four-point scale. The individual is considered non-autistic 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. Based on the administration of multiple assessments, insights into various aspects of autism are gained. We added the Arabic version of Autism Behavior Checklist (ABC). This checklist was initially translated from English into Arabic. A back-translation was then carried out by a professional proficient in the English language. Subsequently, the checklist was given to 5 mothers as a pilot study. This test was administered with the objective of determining whether the translation needed to be adapted; these protocols were not included in the validation study (13). Both Autistic Spectrum Disorder and control groups were subjected to The Questionnaire on Exposure to Heavy Metals, Physical Symptoms, and Child Development (14).

We decided to use Hair mineral analysis to evaluate the long term metal exposure, and urine metal analysis to evaluate immediate exposure. Sample preparation and testing were performed in Germany. ❑

METHODOLOGY OF SAMPLE ANALYSIS

Hair

Samples were collected from the Autistic Spectrum Disorder (ASD) group and the control group. We took hair samples close to scalp from the occipital area for testing, and 10ml of each baseline urine sample. All samples were shipped to Micro Trace Minerals laboratory in Germany were the analysis was performed, following standard analytical procedures. Before testing, samples were repeatedly washed in the laboratory with a de-ionized detergent, rinsed 3 times with de-ionized water and dried in a specially-designated oven before weighing. For sample digestion, certified metal-free acids were used. Digestion took place in a closed-vessel microwave digestion system. Ultrapure water was used for final sample dilution and the analysis was performed via inductively coupled plasma with mass spectrometry (ICP-MS) utilizing collision/reaction cell methods coupled with ion-molecule chemistry, a reliable new method for interference reduction. Certified hair standards and in-house standards were used as part of the laboratory quality control and for the validation of results. Test values were reported in mg/kg (mcg/g).

Urine

Urine samples were collected from all children in the early morning. To avoid contamination, metal-free urine collection cups and tubes had been provided to the Centre by Micro Trace Minerals Laboratories of Germany. At the laboratory, samples were acid-digested with certified metal-free acids involving closed vessel microwave digestion. For sample dilution ultrapure water was used. Testing was performed via inductively coupled plasma with mass spectrometry (ICP-MS) as described above. Certified urine standards and in-house standards were used for quality control and to validate results. To avoid the potentially great margin of error that can result from fluid intake and sample volume, results were reported in mcg/g creatinine for all elements, except calcium, magnesium and zinc. For these elements values were reported in mg/g creatinine.

Statistical analysis

Statistical evaluation of data included a comparison of test values to existing reference ranges for children as reported by the Umweltbundesamt Germany (Environmental Protection Agency). For those metals for which no official reference ranges exist, statistical ranges were established by following standard laboratory procedures. All test data was converted and manipulated by using Spss software program version 17.0. Data was analyzed, mean and standard deviation was estimated as regarding age, developmental mile stones and heavy metal and trace elements levels either in hair or urine comparing the ASD group with controls. The t test was applied and p value was established to determine the statistically significant difference between the two groups. While numbers and percents were calculated among cases and controls, the presence of certain problems, either intrauterine or during infancy, non parametric tests for statistical significant difference was applied comparing case and control groups. The two groups were consid-

Variable	Case (n=25)	Control (n=25)	p value
Age	5.29±1.9	6.25±2.31	0.08
Mothers' age	34.40±6.58	28.12±6.32	0.001**
Sex			
Boys	22 (88.0%)	19 (76.0%)	0.46
Girls	3 (12.0%)	6 (24.0%)	
Developmental mile stones			
Sitting	7.0±0.94	6.6±0.96	0.14
Crawling	10.8±1.57	10.6±1.93	0.7
Walking	13.7±1.49	13.52±1.16	0.6
Talking	12.8± 4.39 (n=10)	11.2±1.76	0.000**
	22.45+ 5.43	(n=25)	
Regression	(n=11)		

TABLE 1. Characteristics of the participants

ered statistical significant when $p<0.05$, and considered highly statistical significant when $p<0.01$. ❑

RESULTS

Characteristics of the participants:

Table 1 shows that the autistic group consisted of 22 boys (88%) and 3 girls. Their mean age was 5.29±1.9. The control group was 19 (76%) boys and 6 (24%) girls with the mean age of 6.25±2.31. There was a significant difference between the mothers' age of autistic group versus the control children ($p=0.001$). There was no significant difference regarding the developmental milestones apart from talking. Only 10 autistic children with the mean age of 12.8±4.39 could produce words in comparison to 25 control children with the mean age of 11.2±1.76 ($p=0.000$). The average mean age of regression in 11 autistic children was 22.45±5.43.

There were significant differences regarding the amount of sea food eaten per month by mothers during pregnancy ($p=0.023$) and by infants in the infancy period ($p=0.011$). Also, mothers of autistic children were exposed to second hand smoke more than mothers of the control children with significant difference ($p=0.04$). There were no significant differences regarding exposure to other environmental risks in pregnancy as maternal exposure to Rhogam shot, vaccination, painting, smoke, second hand smoke, contact lenses, tattoos and pesticides. There were no significant differences in infancy regarding negative reaction to vaccination, lick paint and eating non-food items.

There were no significant differences regarding the physical symptoms that may result from exposure to environmental risk factors as GIT problems, sleep problems, low muscle tone, salivation, and thrush.

There were statistically significant differences in the mean hair levels of arsenic, cadmium,

Environmental exposure		Cases (25)	Control (25)	p value
Seafood taken (during pregnancy) /month		4.12±1.9	3. 0±1.4	0.023*
Duration of breast fed (infancy)		13.16±7.3	14.5±5.5	0.46
Seafood (during infancy)/month		4.64±1.7	3.28±2.0	0.011*
Rhogam shot (pregnancy)	No	24 (96.0%)	21 (84.0%)	0.15
	Yes	1 (4.0%)	4 (14.0%)	
Vaccination (pregnancy)	No	17 (68.0%)	16 (64.0%)	0.5
	Yes	8 (32.0%)	9 (36.0%)	
Painting (pregnancy)	No	15 (60.0%)	14 (56.0%)	0.5
	Yes	10 (40.0%)	11 (44.0%)	
Smoke (pregnancy)	No	17 (68.0%)	19 (79.2%)	0.3
	Yes	8 (32.0%)	5 (20.8%)	
Second hand smoke (pregnancy)	No	9 (36%)	16 (64.0%)	0.04*
	Yes	16 (64.0%)	9 (36.0%)	
Contact lenses (pregnancy)	No	22 (88.0%)	25 (100.0%)	0.1
	Yes	3 (12.0%)	0 (0.0%)	
Prenatal supplements (pregnancy)	No	10 (40.0%)	7 (28.0%)	0.28
	Yes	15 (60.0%)	18 (72.0%)	
Tattoos (pregnancy)	No	19 (76.0%)	22 (88.0%)	0.23
	Yes	6 (24.0%)	3 (12.0%)	
Pesticides (pregnancy)	No	5 (20.0%)	5 (20.0%)	0.37
	Yes	20 (80.0%)	20 (80.0%)	
Negative reaction to vaccination (infancy)	Mild	18 (72.0%)	14 (56.0%)	0.19
	Moderate	7 (28.0%)	9 (36.0%)	
	Severe	0 (0.0%)	2 (8.0%)	
Frequent lick paint (infancy)	Never	10 (40.0%)	9 (36.0%)	0.9
	Mild	10 (10.0%)	12 (48.0%)	
	Moderate	4 (16.0%)	4 (16.0%)	
	Severe	1 (4.0%)	0 (0.0%)	
Frequent eating non food (infancy)	Never	9 (36.0%)	7 (28.0%)	0.4
	Mild	12 (48.0%)	11 (44.0%)	
	Moderate	3 (12.0%)	7 (28.0%)	
	Severe	1 (4.0%)	0 (0.0%)	

TABLE 2. Environmental exposure in both autistic and control groups in pregnancy and infancy periods

barium, cerium and lead (p=0.01, 0.03, 0.003, 0.003, and 0.03 respectively). There were statistically significant differences in the mean urine levels of aluminum, barium, cerium, mercury, and lead (p=0.004, 002, 0.014, 0.006 and 0.004 respectively).

There were statistically significant differences in the mean hair levels of magnesium and zinc (p=0.001 and 0.003 respectively). There were statistically significant differences in the mean urine levels of copper and germanium (p=0.049 and 0.02 respectively). ❑

DISCUSSION

We conducted the study to examine the possible risk factors and sources of exposure to mercury and other heavy metals in children with autistic spectrum disorder, and assessed the levels of trace elements and heavy metals in hair and urine of both autistic and control groups. Our evaluation determined the following risk factors for the development of the autistic spectrum disorder:

1. Eating sea food during pregnancy and infancy periods
2. Second hand smoke exposure during pregnancy
3. Advanced age of the expecting mother

Our results indicated that the mean age of the mothers at the birth of their autistic children was 34.40±6.58 years, versus 28.12±6.32 years for the mothers of the typical children. The difference was statistically significant and may denote that advanced maternal age could be a risk factor of ASD. Croen et al. were in agreement with our result as they concluded that advanced maternal and paternal ages are independently associated with ASD risk (15). The mothers of autistic children reported fewer injections with Rhogam than the mothers of the control group. This was in agreement with Adams et al. (16) and in marked contrast to the study by Holmes et al. (17) that reported on a high use of Rhogam in mothers of children with ASD. It is possible that the difference is partially due to a sampling bias, since the Holmes' study involved her patients, some of whom knew of her interest in Rhogam before becoming pregnant. Regarding maternal seafood consumption during pregnancy, it was found that mothers of children with ASD reported consuming 4.12±1.9 servings of seafood per month, versus 3.0±1.4 servings in mothers of typical children with significant difference. Also, children with ASD were consuming 4.64±1.7 servings of sea food per month in comparison of 3.28±2.0 servings per month in control children with significant difference. This result can explain the significant increase of the mean of urinary mercury excretion 2.48±2.34 that may denote acute intoxication by taking seafood. Adams et al. were in agreement with our results as they found that 57% of mothers of children with ASD reported consuming more than 2 servings of seafood per month, vs. only 33% of mothers of healthy children. Mothers of children with ASD nursed their children for approximately the same length of time as did the mothers of control-group children. Sixteen

Physical Symptoms		Cases (25)	Control (25)	p value
GIT problems	Never	7 (28.0%)	7 (28.0%)	0.4
	Mild	6 (24.0%)	12 (48.0%)	
	Moderate	11 (44.0%)	3 (12.0%)	
	Severe	1 (4.0%)	3 (12.0%)	
Sleep problems	Never	3 (12.0%)	4 (16.0%)	0.9
	Mild	14 (56.0%)	12 (48.0%)	
	Moderate	3 (12.0%)	4 (16.0%)	
	Severe	5 (20.0%)	5 (20.0%)	
Low muscle tone	Never	13 (52.0%)	10 (40.0%)	0.3
	Mild	8 (32.0%)	8 (32.0%)	
	Moderate	3 (12.0%)	5 (20.0%)	
	Severe	1 (4.0%)	2 (8.0%)	
Salivation/drooling	No	16 (64.0%)	17 (68.0%)	0.77
	Yes	9 (36.0%)	8 (32.0%)	
Thrush (yeast infection)	Never	15 (60.0%)	13 (52.0%)	0.7
	Mild	6 (24.0%)	8 (32.0%)	
	Moderate	2 (8.0%)	4 (16.0%)	
	Severe	2 (8.0%)	0 (0.0%)	

TABLE 3. Physical symptoms affection in autistic and control group

mothers (64%) of ASD children were exposed to second hand smoke more than mothers of controls (total of 9 = 36%), indicating a significant difference. This agrees with Zhang et al. (18) who found that 18.9% of autistic mothers were exposed to second hand smoke in contrast with 6.3% in controls. Bilder et al. (19) concluded from their study that second hand smoke is considered the most prominent risk factor in prenatal exposures in mothers of autistic children. Hultman et al. (20) confirmed that daily smoking in early pregnancy was also reported to be associated with an increased risk of ASD. On the other hand, Larson et al. (21) was not in agreement as he found that no statistically significant associations was found between autism and smoking reported at the first antenatal visit. Also, mothers of autistic children showed more exposure to smoking, tattoos, and pesticides than the mothers of the control group, but we found no significant differences in infancy regarding negative reaction to vaccination. We also found no significant difference in paint licking or eating non-food items that may contribute to metal toxicity. These results were in contrast with other studies as Geier et al. (22) stated that some possible sources of heavy metal poisoning include mercury-containing vaccines.

If we consider multiple toxic exposure one contributing factor that triggers ASD, mothers attempting pregnancy be should be diagnostically evaluated for heavy metal exposure prior to conception. To our knowledge, such diagnostic evaluations have not been considered among physicians or obstetricians, but such simple and inexpensive tests may be incorporated into standard medical care to prevent ASD and other diseases linked to metal overexposure.

We did not perform metal testing on the mothers of the autistic or control group, but recommend additional studies to that effect.

Assessment of physical symptoms showed no significant difference between the autistic and the control group. Only 11 (44%) autistic

	Hair values in mg/kg = mcg/g			Urine values in mcg/g creatinine -		
Heavy Metal	Cases (N=25) Mean±SD	Control (N=25) Mean±SD	p value	Cases (N=25) Mean±SD	Control (N=25) Mean±SD	p value
Silver	0.31± 0.33	0.39 ± 0.61	0.57	0.30± 0.33	0.26 ± 0.28	0.68
Aluminum	8.89 ±6.16	7.03±5.09	0.25	111.26 ±142.94	22.98±21.29	0.004**
Arsenic	0.20 ± 0.26	0.06 ± 0.04	0.01*	37.58 ± 30.12	32.06 ± 45.26	0.61
Boron	0.83±0.68	0.68±0.62	0.40			
Barium	1.81±0.9	0.38±0.26	0.03*	7.93±6.59	3.13±3.36	0.002**
Bismuth	0.02 ± 0.02	0.04±0.06	0.13			
Beryllium	0.0001 ±0.0003	0.00±0.0002	0.6	0.67 ±1.44	0.28±0.35	0.2
Cadmium	0.23±0.27	0.06±0.5	0.003**	0.41±0.26	0.53±0.38	0.21
Cerium	0.004±0.005	0.01±0.01	0.003**	2.32±4.26	0.12±0.53	0.014*
Gallium	0.02±0.04	0.02±0.02	0.97	0.29±0.32	0.29±0.30	0.98
Mercury	0.47±0.42	0.30±0.31	0.11	2.48±2.34	1.10±0.63	0.006**
Nickel	0.55±0.83	0.58±0.73	0.89	13.63±12.34	12.60±10.25	0.75
Lead	0.01±0.02	0.01±0.01	0.03*	8.45±7.33	3.36±4.11	0.004**
Palladium	0.01±0.002	0.01±0.01	0.91	1.75±1.59	1.49±1.08	0.50
Antimony	0.08±0.11	0.07±0.06	0.69	0.48±0.65	0.21±0.19	0.051
Platinum	0.0±0.0	0.01±0.05	0.33	0.12±0.14	0.11±0.11	0.92
Tin	0.37±0.25	0.36±0.29	0.84	2.76±4.49	2.28±3.75	0.68
Titanium	1.68±0.22	0.56±0.25	0.08	19.16±13.74	18.88±14.51	0.94
Thalium	0.001±0.002	0.0001±0.0003	0.36	0.70±1.98	0.27±0.26	0.29
Uranium	0.02±0.01	0.02±0.01	0.33	0.06±0.10	0.02±0.07	0.16
Vanadium	0.07±0.05	0.1±0.14	0.34			
Tungsten	0.004±0.005	0.003±0.0006	0.68	16.34±80.14	1.27±5.47	0.35
Zirconium	0.13±0.4	0.06±0.09	0.40	0.81±1.76	1.03±0.49	0.92

TABLE 4. Comparison between mean levels of toxic metals in hair and urine of both autistic and control groups

children showed moderate GIT affection in contrast to 3 (12%) of controls. In contrast to Adams et al. (23) who noted that physical symptoms including gastrointestinal, and sleep problems, low muscle tone and drooling were present in ASD children with a high statistical significance.

Toxic metals in hair and urine

Our findings indicate a significant differences between autistic and control children in the mean hair levels of arsenic, cadmium, barium, cerium and lead (p=0.01, 0.03, 0.003, 0.003, and 0.03 respectively).

Arsenic

While the arsenic concentration was significantly higher in the hair of the autistic children, the urinary excretion of arsenic was not significantly different. This may indicate that long term exposure to even small amounts of arsenic may affect autistic children more than healthy children. Indeed, higher fetal and infant mortality, developmental delay, diminished intellectual ability and attention deficit disorders have been associated with high arsenic levels in water (24,25).

Cadmium

Compared to the healthy group, hair cadmium concentrations were significantly higher in the autistic. However, urinary excretion levels were higher in healthy children, which may indicate that cadmium accumulation in tissue affects the autistic more than healthy children. Cadmium is a cell toxin, and high level of cadmium is found in shellfish, art supplies, cigarette smoke, processed food, fertilizers, fresh water fish, and batteries (26). Cadmium is known to interrupt hormone functions (27).

Mercury and lead

Compared to the control group, hair and urine mercury levels were higher in the autistic group, indicating that past and immediate exposure is greater in the autistic group. Seafood consumption may be the cause. Lead levels were also significantly higher in the hair and urine of the autistic, suggesting that immediate and long term exposure is greater in the autistic than in healthy children. Mercury and lead have been associated with neurological disorders (28,29).

Pyria's study showed a significant elevation in the levels of toxic metals Pb and Hg in hair of

	Hair values in mg/kg (=mcg/g)			Urine values in mcg/g creatinine- unless noted otherwise		
Trace element	Cases (N=25) Mean±SD	Control (N=25) Mean±SD	p value	Cases (N=25) Mean±SD	Control (N=25) Mean±SD	p value
Cobalt	0.04±0.05	0.03±0.04	0.38	1.76±1.46	3.06±3.18	0.07
Calcium	311.51 ±169.73	320.49 ± 183.15	0.86	85.27 ±69.63 mg/g crea	185.80 ±271.34 mg/g crea	0.08
Chromium	0.09±0.06	0.10±0.09	0.64	2.13±2.02	1.78±2.73	0.6
Cesium				11.11±8.62	10.34±9.38	0.76
Copper	21.94±21.7	19.69±9.95	0.64	53.25±43.70	32.63±29.49	0.049*
Iron	10.89±4.28	11.02±5.33	0.93	42.24±58.10	37.54±36.91	0.73
Germanium	0.006±0.005	0.008±0.009	0.42	1.01±0.84	0.54±0.40	0.02*
Iodine	2.50±8.49	0.58±0.5	0.27	280.16±336.98	645.71±468.99	0.69
Lithium	0.002±0.004	0.002±0.004	0.98	34.34±34.91	28.67±20.17	0.49
Magnesium	22.4±12.5	70.21±69.43	0.001**	328.43±236.81 mg/g crea	278.68±193.30 mg/g crea	0.42
Manganese	0.38±0.24	0.41±0.34	0.71	7.32±7.22	4.81±3.67	0.13
Molybdenum	0.08±0.1	0.06±0.03	0.35	1277.34±3864.72	123.50±56.51	0.14
Strontium	0.86±1.38	0.78±0.4	0.81	142.38±404.72	81.77±102.06	0.47
Selenium	0.80±0.25	0.36±0.29	0.28	286.18±236.75	311.17±254.15	0.76
Vanadium				1.61±2.68	0.98±0.78	0.27
Zinc	101.042±52.0	149.86±58.51	0.003**	22.13±106.22 mg/g crea	0.89±0.45 mg/g crea	0.32

TABLE 5. Comparison between mean levels of trace elements in hair of both autistic and control groups

autistic children when compared to healthy control group (30).

Multiple Metal Exposure

Fido and AlSaad in Kuwait found that the children with autism had significantly higher in-hair concentration levels of lead, mercury and uranium (31). Al-Ayadhi reported that hair samples from autistic patients and children with Aspergers syndrome living in Riyadh contained significantly higher concentration of the toxic heavy metals mercury, lead, arsenic, antimony and cadmium (32). El Sheshtawy reported highly significant differences between ASD cases and controls in an Egyptian sample (33). The hair level of lead was significantly higher in autistic cases than in controls. The level of copper was significantly higher in the autistic than in controls. The level of zinc was significantly lower in autistic cases than in controls.

The result of our study revealed statistically significant differences in the mean urine levels of Aluminum, Barium, Cerium, Mercury, and Lead ($p=0.004$, 002, 0.014, 0.006 and 0.004 respectively) which confirms results of other studies, and maybe linked to seafood consumption. Bradstreet's case-control study found that children with autism excreted 3.1 times more mercury into their urine (34) and Adams et al. documented that urine samples of autistic children showed extremely high baseline aluminum (63× average of other participants), antimony (45× average), bismuth (40× average), cadmium (7× average), lead (12× average), tin (12× average) and uranium (65× average) (35). Furthermore, Al-Ayadhi reported that hair samples from autistic children in Riyadh contained significant lower concentration of the following essential trace elements: calcium, copper, chromium, manganese, magnesium, iron, selenium and cobalt (36).

It may be argued that children with autism spectrum disorder are not the only children exposed to potentially toxic metals. The reason autistic patients show greater concentration of potentially toxic metals in tissue may be the result of a greater ability to accumulate toxins, which in turn leads to an alteration of biochemical processes. Our data supports this point. In our study, the children with autistic spectrum disorders displayed lower levels of the nutritional elements calcium, copper, chromium, manganese, magnesium, iron, selenium and cobalt. Since autistic children display poor eating habits, the low tissue levels may be explained by an inadequate nutritional intake. The ability of selenium compounds to modify profoundly the toxicity of both organic and inorganic mercury compounds has been demonstrated in experimental animals by Parizek and co-workers as early as 1971 (37). Iron deficiency and lead poisoning are common among infants and children in many parts of the world, and often these two problems are associated. Sufficient iron stores may reduce the risk of lead poisoning (38). Through a pharmacokinetic analysis of lead absorption, distribution, and elimination in calcium-deficient rats, Aungst and Fung observed changes in lead absorption and systemic clearance associated with the calcium-deficient diet the animals were placed on. Calcium deficiency could elevate blood lead accumulation and thus potentially influence susceptibility to lead toxicity (39). Goyer reports that iron deficiency increases absorption of cadmium, lead, and aluminum, and lead interacts with calcium in the nervous system to impair cognitive development (40).

Trace elements in hair and urine

1. Hair

Important minerals and nutrient that have significant neurological and health effects are magnesium, lithium, zinc, iron, vitamin B6, B1, B12. There were statistically significant differences in the mean hair levels of magnesium and zinc ($p=0.001$ and 0.003 respectively). This was in agreement with Priya and Geetha who found that magnesium and selenium levels were significantly decreased ($p<0.001$) in autistic children when compared to controls (41). In our study, hair zinc levels were significantly lower in the autistic group when compared to the control group. A group of autistic subjects averaging 5.7 years of age [$n=7$] showed significantly lower hair levels of calcium, magnesium, chromium, copper, manganese, and lithium, but similar levels of mercury compared to controls. They also showed significantly lower selenium levels and a trend toward a lower zinc status. A study by Jory and Mc Gerris found lower red-cell selenium in children with autism versus age-matched controls (42).

2. Urine

For the essential elements, the autistic group showed statistically significant differences in the mean urine levels of copper and germanium ($p=0.049$ and 0.02 respectively), and a moderate initial increase of excretion of man-

ganese and vanadium which may be diet related. Manganese is found in relatively high amounts of tea; vanadium is widely distributed in food such as fish, olives, and vegetable oils. Since urine is not an ideal specimen to substantiate deficiency, these values and evaluations seem insignificant.

Limitation of our study: Sample size

A larger sample size, from multiple sites, is needed to improve the statistical power of this study and validate or refute its findings. Sample bias for controls: The controls were chosen from the friends and neighbors of the autistic patients. This allowed an easy access to a reasonable match of geographic location and socio-economic status, but is not the most rigorous method. Metal testing on the mothers of the ASD and control groups might have provided additional answers into the cause and onset of the disease, and we recommend further studies to evaluate this avenue. ❑

CONCLUSION

Our findings support several prenatal and infantile risk factors for autism. Using 25 children with autism and 25 gender and age matched controls in K.S.A. We identified multiple toxic metal exposure associated with autism. Maternal second-hand smoke exposure and maternal and infantile seafood consumption can be associated with autism.

The present study demonstrated toxic metal exposure and nutritional inadequacies in children with autistic spectrum disorders as compared to normal children, suggestive of a possible pathophysiological role of heavy metals and trace elements in the genesis of symptoms of autism spectrum disorders. The poor eating habits of autistic children are considered one cause of the nutritional inadequacies located through trace element testing.

In a more highly contaminated environment the diagnosis of heavy metal exposure should become standard practice. While hair mineral analysis is considered controversial, the validity of test results can, and should be confirmed through further investigation methods such as blood and urine analysis. Since every metal test (serum, whole blood, urine or hair) provides specific information about each metal's pathway including a metal's effect on body chemistry and symptom development, careful interpretation of diagnostic results can provide valuable information regarding the type and extend of metal exposure. All this can lead to treatment methods aimed at reducing toxic exposure. Our study suggests that autistic children are environmentally more threatened than normal children. Reducing toxic exposure should be the aim of pediatricians caring for ASD and other children. Further studies may indicate that such a treatment approach is best extended to, or started with females attempting pregnancy.

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