

# Toxic Metals and Essential Elements in Hair and Severity of Symptoms among Children with Autism

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## ABSTRACT

**Objective:** The objective of this study was to assess the levels of ten toxic metals and essential elements in hair samples of children with autism, and to correlate the level of these elements with the severity of autism.

**Method:** The participants were 44 children, age 3 to 9 years, with Autistic Spectrum Disorder (ASD) according to Diagnostic and Statistical Manual of Mental Disorders 4<sup>th</sup> Edition, (DSM-IV). The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS). Hair analysis was performed to evaluate the long term metal exposure and mineral level.

**Results:** By comparing hair concentration of autistic vs nonautistic children, elevated hair concentrations were noted for aluminum, arsenic, cadmium, mercury, antimony, nickel, lead, and vanadium. Hair levels of calcium, iron, iodine, magnesium, manganese, molybdenum, zinc, and selenium were considered deficient. There was a significant positive correlation between lead & verbal communication ( $p = 0.020$ ) and general impression ( $p = 0.008$ ). In addition, there was a significant negative correlation between zinc & fear and nervousness ( $p = 0.022$ ).

**Conclusion:** Our data supports the historic evidence that heavy metals play a role in the development of ASD. In combination with an inadequate nutritional status the toxic effect of metals increase along with the severity of symptoms.

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## INTRODUCTION

**A**utistic spectrum disorder (ASD) is a neurodevelopmental syndrome with onset prior to age 36 months. Diagnostic criteria consist of impairments in sociality and communication plus repetitive and stereotypic behaviour (1). Traits strongly associated with autism include movement disorders and sensory dysfunctions (2). Although autism may be apparent soon after birth, most autistic children experience at least several months, even a year or more of normal development, followed by regression and defined as loss of function or failure to progress (3).

Previous research suggested that mercury and other toxins play a role in the pathogenesis of autism (1,3). Similarly, the lack or excess of essential minerals is known to cause a variety of health problems, and could contribute to the etiology of autism. The diagnoses of metal intoxication or a mineral/trace element deficiency can be assessed by blood, urine, and hair (4). ASD children are a considerable challenge when it comes to blood drawing, even urine collection is difficult. Hair mineral analysis is easy and painless, and research suggests its usefulness as an early predictor of toxic exposure. The EPA stated that hair is "a meaningful and representative tissue" for measuring toxic metals and selected nutrients. Similarly, the National Health and Nutrition Evaluation Study continue to use hair as one method to evaluate levels of metals such as mercury (5,6).

Compared to adults, children tend to be more exposed to environmental toxins as a consequence of their behaviors, have higher absorption rates, and lower detoxification ability (7). Through a pilot study, Lonsdale et al confirmed the relation between heavy metal toxicity and neurobehavioral disorders (8). By evaluating hair and nail samples of autistic children, Priya et al could correlate an excessive body burden of copper with the severity of ASD (9).

The biological effects of metals are linked to their chemical properties (10), and it appears that excess metal exposure may be a common source of neurotoxicity in multiple populations around the world (11). □

## AIM OF THE WORK

**T**his study was designed to investigate the hypothesis that children with autism show abnormal levels of toxic metals, or are deficient in essential elements (12-14). Through hair mineral analysis we opted to confirm or dismiss this.

Another purpose of this study was to evaluate whether the level of these elements could be correlated with the severity of autism. □

## SUBJECTS AND METHODS

**T**he participants were 44 Autistic Spectrum Disorder (ASD) children (37 boys and 7 girls) between the age of 3 and 9 years. A total of 39 children were diagnosed as autistic; two children were diagnosed as Asperger Syndrome, and three with PDD NOS (pervasive developmental disorder, not otherwise specified). The children had been 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 and 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 4<sup>th</sup> Edition, (DSM-IV) (15).

The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS) translated by El-Dafrawi (16). This rating scale 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.

All of the children admitted received routine childhood vaccinations.

### Exclusion criteria:

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

**Entry criteria:**

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

Metals circulate in the blood stream, but blood sampling of an autistic population is a considerate challenge to patients and medical personal. Research suggests the usefulness of hair analysis (17). Hair is tissue, and nourished by the blood stream. As long as metals circulate in the blood stream, they can be stored in body tissue, hair included. Samples are easily available. Since sampling is easy and painless. We decided to use hair analysis to evaluate the long term metal exposure as it relates to the Autistic Spectrum Disorder.

Research provides evidence that metals and metalloids in scalp hair can indicate increased exposure (18). Razagui and Haswell reported that peripheral biological tissue, such as hair provides supporting evidence of neonatal metal exposure (19). Yorbik et al demonstrated that the hair zinc status of autistic children was low when compared to normal Turkish children (20) and in 2007, Adams et al tested baby teeth of autistic children and noted that a higher body burden of mercury than controls (21). Nnorom et al demonstrated that multi elemental analysis of hair reflects metal exposure (22).

Hair samples were collected of all participants (including the control group which provided our reference ranges) during the period of June 2006 to September 2010. All parents signed informed consent forms.

**Methodology of Hair Sample Analysis**

Samples were collected from the Autistic Spectrum Disorder (ASD) group of 44 children. We took hair samples close to scalp from the occipital area for testing. Samples were shipped to Micro Trace Minerals laboratory in Germany where the analysis was performed.

Before testing, samples were repeatedly washed in the laboratory with a metal-free detergent, rinsed 3 times with ultrapure water and dried in a special drying 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 elemental analysis was performed via inductively coupled plasma 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.

**Statistical Analysis**

We computerized special data files, using Excel program 2010. Data of all 44 participants were converted by using SPSS software program version 17.0, analyzing characteristics of samples. We calculated mean values and standard deviation in regard to the patient's age (in months) and developmental mile stones.

We correlated the number of all 44 participants into percentages, calculated sex distribution and patient groups in regard to diagnosis and total CARS, and categorized them into three groups: mild, moderate and severe. We calculated the number and percentage of autistic children with levels above range, either in toxic minerals or in essential minerals and trace elements. Similarly, we calculated the number and percentage of autistic children showing levels below the reference range for essential minerals and trace elements.

As a reference range or control range, we evaluated hair analysis results from a non-autistic group of children who were of the same age group. The total control group consisted of 146 children, randomly selected. Of these, 32 were females (22%) and 114 (78%) were male, a percentage comparatively similar to that of our autistic test group where 84% are males and 16% were females (see Table 1).

For the test group, we calculated the mean and standard deviation for each metal or mineral and trace element. We calculated the p value, and correlated the toxic metals and essential elements with subscale and total scores of CARS to test the positive and negative relations between the two variables. The data was considered statistically significant when  $p < 0.05$ . It was considered statistically highly significant when  $p < 0.01$ .

**RESULTS**

Table 1 shows that 37 (84%) of the sample came from boys in contrast to 7 (16%) girls. The mean age of the entire test group (male and female) was  $5.11 \pm 1.57$ . Thirty-nine (88.6%) of the 44 children tested had been diagnosed as autistic, 2 (4.5%) with Asperger Syndrome, and 3 (6.9%) with pervasive developmental disorder PDD (NOS). The mean age in months for sitting was  $6.77 \pm 0.96$ , for crawling  $10.41 \pm 1.65$ , and for walking  $13.70 \pm 1.49$ . Only 15 of the test persons (34%) had language development at the mean age of  $12.56 \pm 4.03$  months.

**Level of Potentially Toxic Metals in the Hair of Autistic Children**

As shown in Table 2, the mean hair metal concentration was high for aluminum ( $15.21 \pm 9.0$ ), arsenic ( $2.94 \pm 4.05$ ), cadmium ( $0.62 \pm 0.19$ ), mercury ( $3.35 \pm 4.80$ ), antimony ( $0.58 \pm 0.09$ ), nickel ( $2.37 \pm 1.28$ ), and lead ( $4.56 \pm 1.40$ ). Of the 44 children, 13.5 showed vanadium values above the reference range. None of the children showed conspicuous values for uranium and the potentially toxic trace elements chromium.

		Number	Percentage	Mean	SD
Total participants		44	100		
Male		37	84		
Female		7	16		
Age(years)				5.11	1.57
Diagnosis	Autism	39	88.6		
	Asperger Syndrome	2	4.5		
	PDD (NOS)	3	6.9		
Developmental milestones (in months)	Sitting			6.77	0.96
	Crawling			10.41	1.65
	Walking			13.70	1.49
	Talking	15	34	12.56	4.03
Total CARS (mild-moderate)		20	45.5		
CARS (severe)		19	43.2		

TABLE 1. Sample Characteristics.

	No. of autistic children above reference range (RR)	Percent of autistic children above RR	Mean value of hair concentration of autistic children mg/kg	Standard Deviation of mean of all hair tests from autistic children	Hair Reference Range of nonautistic children in mg/kg
Aluminum (Al)	24	54.5	15.21	9.0	8.00
Arsenic (As)	4	9.1	2.94	4.05	0.7
Cadmium (Cd)	7	15.9	0.62	0.19	0.32
Chromium (Cr)	0	0.0	0.08	0.06	0.53
Mercury (Hg)	14	31.8	3.35	4.80	0.5
Antimony (Sb)	1	2.3	0.58	0.09	0.4
Nickel (Ni)	4	9.1	2.37	1.28	0.85
Lead (Pb)	26	59	4.56	1.40	3.00
Uranium (U)	0	0.0	0.02	0.01	0.1
Vanadium (V)	6	13.6	0.12	0.11	0.15

TABLE 2. Number of autistic children exceeding the reference range\* and mean level of hair toxic metal.

\* The reference range is a statistical evaluation of a non-autistic group (N=146) of children, representing a 95th percentile, serving as the control group.

**Level of essential Minerals and Trace elements in the Hair of Autistic Children**

Considered deficient were the elements calcium ( $43.89 \pm 11.75$ ), iron ( $5.70 \pm 1.32$ ), iodine ( $3.55 \pm 12.37$ ), magnesium ( $12.38 \pm 4.49$ ), manganese ( $0.045 \pm 0.007$ ), zinc ( $67.04 \pm 23.78$ ), and selenium ( $0.03 \pm 0.0$ ). The essential trace elements molybdenum was within the reference range for all children except one. The hair concentration of the nonessential trace element lithium was considered normal in all tests. The level of copper ( $133.68 \pm 115.47$ ) was exceeding the upper 95th percentile reference range (Table 3).

**Correlation of Toxic Metal Concentration in Hair with the Subscale and Total Score of CARS**

As shown in Table 4, we noted the following:

- a significant positive correlation between the heavy metal lead, verbal communication ( $p = 0.020$ ) and general impression ( $P = 0.008$ ). This indicates that a higher lead concentration in hair is associated with impaired verbal communication and general impression.
- a significant positive correlation between mercury, Object use ( $p = 0.040$ ) and auditory response ( $p = 0.021$ ). This indicates that a high mercury concentration in hair tissue is associated with impaired object use and auditory response.

- a significant positive correlation between chromium and taste/smell responses ( $p = 0.024$ ), verbal communication ( $p = 0.026$ ) and general impression ( $p = 0.033$ ). This might indicate that elevated chromium in hair is associated with impairment in taste, smell responses, verbal communication and general impression.
- a significant positive correlation between nickel & auditory response ( $p = 0.015$ ), fear and nervousness ( $p = 0.005$ ), non-verbal communication ( $p = 0.009$ ) and Total CARS ( $p = 0.049$ ), demonstrating that higher nickel levels in hair are associated with impaired auditory response, increased fear and nervousness, impaired non verbal communication and higher Total CARS.
- a significant positive correlation between uranium & verbal communication ( $p = 0.033$ ). This means that the higher uranium is associated with impaired verbal communication.

**Correlation between Essential Minerals and Trace Element Levels in Hair with the Subscale and Total score of CARS**

As shown in Table 5, we noted the following:

- a significant negative correlation between zinc & fear and nervousness ( $p = 0.022$ ), and between zinc & verbal communication.

	Number of autistic children outside the reference range	Percent of autistic children Outside the Reference Range	Mean value of all hair test of autistic children	Standard deviation of mean of all hair tests of autistic children	95 percentile Reference Range of nonautistic children (N=146)
Calcium (Ca)	14	31.8	43.98	11.75	200-850
Copper (Cu)	4	9.1	133.86	115.47	6.7-37
Iron (Fe)	6	13.6	5.70	1.32	7.7-15
Iodine (I)	2	4.5	3.55	12.37	7.9
Lithium (Li)	0	0.0	0.0015	0.003	0.5
Magnesium (Mg <sup>9</sup> )	26	59	12.38	4.49	20-115
Manganese (Mn)	2	4.5	0.045	0.007	0.07-0.72
Molybdenum (Mo)	1	2.3	0.03	0.0	0.03-1.00
Zinc (Zn)	0	0.0	0.02	0.01	0.1
Selenium (Se)	22	50	67.04	23.78	110-227
	1	2.3	0.03	0.0	0.2-3.00

TABLE 3. Number of autistic children outside the reference range and mean level of essential minerals and trace elements in hair.

		As	Pb	Al	Cd	Cr	Hg	Ni	Sb	U	V
Relating to people	R	-0.119	0.108	0.268	0.088	0.049	0.077	0.087	-0.139	0.079	-0.066
	P	0.443	0.484	0.080	0.571	0.772	0.618	0.574	0.367	0.609	0.672
	N	44	44	44	44	44	44	44	44	44	44
Imitation	R	-0.173	-0.060	-0.128	0.233	0.117	-0.050	0.075	0.061	0.096	-0.144
	P	0.263	0.699	0.408	0.128	0.483	0.745	0.627	0.695	0.535	0.352
	N	44	44	44	44	44	44	44	44	44	44
Emotional response	R	-0.151	0.077	-0.020	0.230	0.240	-0.012	0.024	-0.111	0.138	-0.119
	P	0.327	0.620	0.898	0.132	0.148	0.938	0.879	0.473	0.372	0.443
	N	44	44	44	44	44	44	44	44	44	44
Body use	R	-0.178	-0.091	-0.065	0.000	0.170	-0.127	0.076	-0.141	-0.020	-0.108
	P	0.249	0.557	0.674	0.996	0.308	0.411	0.625	0.360	0.896	0.484
	N	44	44	44	44	44	44	44	44	44	44
Object use	R	-0.191	-0.011	0.289	-0.163	0.079	0.265	-0.038	-0.154	0.011	-0.131
	P	0.215	0.943	0.057	0.292	0.636	<b>0.040</b>	0.808	0.319	0.942	0.395
	N	44	44	44	44	44	44	44	44	44	44
Adaptation to change	R	0.038	-0.129	-0.184	0.081	0.249	0.135	0.249	-0.288	0.034	0.124
	P	0.805	0.405	0.231	0.601	0.132	0.381	0.103	0.058	0.829	0.424
	N	44	44	44	44	44	44	44	44	44	44
Visual response	R	-0.098	-0.084	0.115	0.289	0.202	0.045	0.227	-0.124	0.129	-0.073
	P	0.525	0.587	0.459	0.057	0.225	0.773	0.138	0.423	0.405	0.638
	N	44	44	44	44	44	44	44	44	44	44
Auditory response	R	0.042	0.065	0.240	0.176	-0.039	0.036	0.364	-0.105	0.039	0.162
	P	0.784	0.676	0.148	0.253	0.817	<b>0.021</b>	<b>0.015</b>	0.497	0.799	0.295
	N	44	44	44	44	44	44	44	44	44	44
Taste, smell responses	R	-0.163	0.050	-0.262	0.033	-0.366	-0.062	0.046	-0.089	0.157	-0.095
	P	0.290	0.747	0.088	0.832	<b>0.024</b>	0.687	0.766	0.565	0.309	0.540
	N	44	44	44	44	44	44	44	44	44	44
Fear/nervousness	R	-0.190	-0.048	0.247	0.173	-0.135	0.107	.413	0.122	-0.093	-0.094
	P	0.213	0.759	0.106	0.261	0.418	0.487	<b>0.005</b>	0.429	0.549	0.543
	N	44	44	44	44	44	44	44	44	44	44
Verbal communication	R	-0.170	0.350	-0.024	-0.105	0.360	0.202	-0.021	0.022	-0.323	-0.115
	P	0.270	<b>0.020</b>	0.875	0.498	<b>0.026</b>	0.189	0.895	0.887	<b>0.033</b>	0.456
	N	44	44	44	44	44	44	44	44	44	44
Non-verbal communication	R	-0.072	-0.017	-0.029	0.007	0.135	-0.036	0.391	-0.070	-0.004	0.043
	P	0.644	0.913	0.851	0.962	0.419	0.817	<b>0.009</b>	0.651	0.981	0.780
	N	44	44	44	44	44	44	44	44	44	44
Activity level	R	0.104	-0.161	-0.209	-0.080	0.223	0.087	0.069	-0.205	-0.261	0.054
	P	0.501	0.295	0.173	0.605	0.178	0.573	0.658	0.181	0.088	0.727
	N	44	44	44	44	44	44	44	44	44	44
Intellectual response	R	.212	0.216	.217	0.104	0.290	0.186	0.047	0.118	-0.277	0.105
	P	.174	0.158	.138	0.500	0.055	0.233	0.773	0.474	0.071	0.490
	N	44	44	44	44	44	44	44	44	44	44
General impression	R	0.065	0.396	-0.052	-0.119	0.347	0.161	-0.013	-0.211	-0.151	0.006
	P	0.675	<b>0.008</b>	0.735	0.442	<b>0.033</b>	0.296	0.935	0.169	0.329	0.971
	N	44	44	44	44	44	44	44	44	44	44
Total CARS	R	-0.209	-0.103	-0.242	0.177	0.300	0.030	0.299	0.183	-0.035	0.056
	P	0.174	0.505	0.113	0.250	0.067	0.848	<b>0.049</b>	0.234	0.821	0.718
	N	44	44	44	44	44	44	44	44	44	44

TABLE 4. Correlation between Hair Toxic Metal Levels with the Subscale and Total Score of CARS.

P = value of significance; R = correlation factor; N = number of patients.

		Zn	Mn	Mg	Ca	Cu	Fe	I	Li	Mo	Se
Relating to people	R	0.130	0.002	0.157	0.129	0.191	0.062	0.039	-0.357	-0.181	0.023
	P	0.402	0.990	0.309	0.404	0.214	0.691	0.800	<b>0.028</b>	0.246	0.880
	N	44	44	44	44	44	44	44	44	44	44
Imitation	R	0.190	0.173	0.105	0.050	0.147	0.074	-0.053	-0.222	-0.174	0.238
	P	0.216	0.262	0.499	0.746	0.340	0.633	0.731	0.180	0.264	0.120
	N	44	44	44	44	44	44	44	44	44	44
Emotional response	R	-0.029	0.230	0.180	0.093	0.163	-0.046	-0.022	-0.329	0.078	0.211
	P	0.852	0.134	0.243	0.550	0.290	0.766	0.886	<b>0.044</b>	0.617	0.169
	N	44	44	44	44	44	44	44	44	44	44
Body use	R	-0.014	-0.056	-0.010	-0.146	-0.056	-0.235	-0.005	0.091	.049	-.146
	P	0.930	0.719	0.947	0.345	0.720	0.125	0.972	0.587	.755	.344
	N	44	44	44	44	44	44	44	44	44	44
Object use	R	-0.047	-0.224	0.061	-0.152	-0.036	-0.182	-0.045	0.073	-.026	-.180
	P	0.760	0.143	0.696	0.324	0.815	0.236	0.771	0.665	.870	.241
	N	44	44	44	44	44	44	44	44	44	44
Adaptation to change	R	0.187	-0.088	0.213	-0.142	0.092	0.045	0.222	-0.359	-0.142	-0.303
	P	0.224	0.570	0.164	0.358	0.554	0.772	0.148	<b>0.027</b>	0.363	<b>0.045</b>
	N	44	44	44	44	44	44	44	44	44	44
Visual response	R	0.006	0.098	0.243	0.106	0.182	-0.102	0.018	-0.367	0.071	0.109
	P	0.970	0.528	0.112	0.493	0.237	0.509	0.910	0.023	.650	0.482
	N	44	44	44	44	44	44	44	44	44	44
Auditory response	R	-0.134	0.107	0.119	0.057	-0.100	-0.042	-0.161	0.086	-0.040	0.193
	P	0.385	0.488	0.440	0.711	0.517	0.789	0.295	0.607	0.797	0.209
	N	44	44	44	44	44	44	44	44	44	44
Taste, smell responses	R	-0.200	-0.174	-0.132	0.095	-0.008	-0.059	0.134	0.020	0.129	-0.093
	P	0.193	0.259	0.393	0.539	0.957	0.703	0.385	0.907	0.408	0.549
	N	44	44	44	44	44	44	44	44	44	44
Fear/nervousness	R	0.345	0.231	0.223	0.141	-0.028	0.259	-0.125	-0.090	0.127	0.075
	P	<b>-0.022</b>	0.131	0.145	0.363	0.856	0.089	0.417	0.591	0.415	0.628
	N	44	44	44	44	44	44	44	44	44	44
Verbal comm.....	R	0.359	-0.072	0.033	0.055	0.069	-0.074	-0.039	0.084	-0.346	-0.091
	P	<b>-0.017</b>	0.641	0.830	0.725	0.658	0.631	0.800	0.615	<b>0.023</b>	0.558
	N	44	44	44	44	44	44	44	44	44	44
Non-verbal comm.	R	0.208	0.216	0.227	0.104	0.290	0.184	0.045	0.119	-0.278	0.106
	P	0.175	0.159	0.138	0.501	0.056	0.231	0.774	0.475	0.071	0.491
	N	44	44	44	44	44	44	44	44	44	44
Activity level	R	0.078	0.015	0.065	0.054	0.290	-0.014	0.075	-0.047	-0.086	0.034
	P	0.613	0.925	0.676	0.726	0.056	0.929	0.627	0.778	0.583	0.829
	N	44	44	44	44	44	44	44	44	44	44
Intellectual response	R	.215	0.227	.217	0.104	-0.291	0.184	0.047	0.116	0.277	0.105
	P	.175	0.138	.138	0.500	0.055	0.231	0.773	0.473	0.071	0.490
	N	44	44	44	44	44	44	44	44	44	44
General impression	R	-0.174	-0.068	-0.028	0.020	0.237	-0.018	0.025	0.155	-0.534	-0.120
	P	0.260	0.660	0.859	0.899	0.122	0.908	0.874	0.353	<b>0.000</b>	0.438
	N	44	44	44	44	44	44	44	44	44	44
Total CARS	R	0.214	0.072	0.220	-0.022	0.124	0.053	0.067	-0.399	-0.118	-0.032
	P	0.162	0.643	0.150	0.888	0.424	0.732	0.664	<b>0.013</b>	0.452	0.838
	N	44	44	44	44	44	44	44	44	44	44

TABLE 5. Correlation between Hair Trace Element Levels with the Subscale and Total score of CARS.

tion ( $p = 0.017$ ). This means that lower hair zinc concentrations are associated with fear, nervousness and more impairment in verbal communication.

- a significant negative correlation between lithium & relating to people ( $p = 0.028$ ), emotional ( $p = 0.044$ ), adaptation to changes ( $p = 0.027$ ), visual response ( $p = 0.023$ ) and Total CARS ( $p = 0.013$ ). This means that a lower lithium hair value is associated with more impairment in relation to people, emotion, adaptation to changes, visual response and more total CARS.
- a significant negative correlation between molybdenum, verbal communication ( $p = 0.023$ ) and general impression ( $p = 0.000$ ), indicating that low hair molybdenum values are associated with disturbed general impression and verbal communication.
- a significant negative correlation between selenium and adaptation to changes ( $p = 0.045$ ). A lower selenium concentration in hair seems to be associated with a disability to adapt to changes. □

## DISCUSSION

The majority of autistic patients in this study were boys (84%) as demonstrated in Table 1. This was in line with Whiteley et al who noted a greater preponderance of males over females (approximately 4:1) among autistic children (23).

The mean age of the whole sample was  $5.11 \pm 1.57$  (Table 1). This was consistent with Pervasive Developmental disorder which according to the American Psychiatric Association appears to affect children at the age of 3 to 10 years (24). Thirty-nine children were diagnosed as autistic, 2 (4.5%) as Asperger Syndrome, and 3 (6.9%) were diagnosed as PDD (NOS) (Table 1). This was consistent with Fombonne who claimed that autism is the most common of the Pervasive Developmental Disorders (25).

Filipek et al. stated that in some cases, autistic infants appear to develop normally until age 1 to 3 years, then sudden changes occur that indicate the presence of ASD (1). Our study suggests that toxic metal exposure in combination with an inadequate nutritional status is a likely cause.

Our data supports the historic evidence that heavy metals, especially lead play a role in the development of ASD. As early as 1976, Cohen et al. noted elevated blood lead levels in ASD children (26). In 1998 Kumar et al. confirmed elevated blood lead levels in ASD children (27). Lonsdale et al. observed increases in urinary concentrations of cadmium, nickel, and lead among children with pervasive mental disorder (8). AL-Ayadhi in his evaluation of Riyadh children found significantly higher levels of toxic heavy metals mercury, lead, arsenic, antimony and cadmium in the hair of children with autistic spectrum disorder as compared to normal children (28).

In 2009, Blaylock et al. reported that aluminum causes oxidative stress within brain tissue, exacerbating the clinical presentation of autism by worsening of excitotoxicity and by microglial priming (29). At the same time, a study of Kuwaiti children found significant elevations of environmental metals in children with autism. Mercury levels of the autistic group were 15 times higher than in the control group (30,31).

Mercury is often discussed as a potential cause or aggravator of neurological disease patterns such as ASD. Bernard et al noted that autistic children who were postnatally poisoned developed articulation problems, from slow, slurred word production to an inability to generate meaningful speech (32). We found a significant positive correlation between mercury & Object use ( $p = 0.040$ ) and Auditory response ( $p = 0.021$ ). Our study also indicates that auditory response is affected by nickel ( $p = 0.015$ ) and so is Fear and Nervousness ( $p = 0.005$ ), Non Verbal communication ( $p = 0.009$ ) and Total CARS ( $p = 0.049$ ) and higher Total CARS.

Communication and learning problems are common among ASD patients. Brockel and Cory-Slechta found high lead levels to be associated with negative effects on childhood development, cognitive ability, learning and behavioral disabilities, attention deficit hyperactivity disorder, impulsivity, and inability to inhibit inappropriate responding (33).

Our data supports this. As can be seen in Table 4 Verbal communication ( $p = 0.020$ ) and General impression ( $p = 0.008$ ) are significantly affected by the neurotoxin lead.

Toxic metals affect trace element absorption, and the interaction between essential elements and toxic metals affects threshold values



and toxicity effects (34). The toxic metals cadmium, lead, mercury, and aluminum may interact metabolically with nutritionally essential metals. Iron deficiency increases absorption of cadmium, lead, and aluminum. Lead interacts with calcium in the nervous system to impair cognitive development. Cadmium and aluminum interact with calcium in the skeletal system to produce osteodystrophies. Lead replaces zinc on hem enzymes and cadmium has the potential to replace zinc. Calcium deficiency along with low dietary magnesium may contribute to aluminum-induced degenerative nervous disease (35). Koziyec investigated the magnesium status of children with ADHD and found magnesium deficiency most frequently in hair (77.6%), compared to serum (33.6%) (36). Vasconcellos et al documented that the mercury/selenium ratio in hair increases with mercury concentration, supporting research that indicates the protective role of selenium (37). While hair mineral analysis alone cannot diagnose acute nutritional deficiencies, hair test values reflect and detect an inadequate nutritional status (38).

In our study, we found deficient levels of calcium ( $43.89 \pm 11.75$ ), iron ( $5.70 \pm 1.32$ ), iodine ( $3.55 \pm 12.37$ ), magnesium ( $12.38 \pm 4.49$ ), manganese ( $0.045 \pm 0.007$ ), zinc ( $67.04 \pm 23.78$ ), and selenium ( $0.03 \pm 0.0$ ). The level of copper ( $133.68 \pm 115.47$ ) exceeded the reference range (Table 3) and this confirms the findings of Faber et al. who reported that the frequency of zinc deficiency and copper intoxication is highly common in children with ASD (39). We could also confirm the research of AL-Ayadhi, stating that compared to normal children, the hair samples from children with autistic spectrum disorders show significantly lower concentration of calcium, chromium, manganese, and iron (28). We supported the findings of Adams et al, indicating that low iodine levels are prevalent among children with autistic disorder and confirmed the likelihood that an inadequate iodine status affects development of speech and cognitive skills (40).

Selenium protects from mercury and methylmercury toxicity (34). Our data showed significantly lower hair selenium levels ( $0.03 \pm 0.0$ ) in children with autism, supporting the work of Jory and Woody who had found low selenium levels in red cells of autistic children (41). We found a significant negative correlation between Selenium & Adaptation to changes ( $p =$

$0.045$ ). Apparently, a low selenium hair concentration is associated with disability to adapt to changes. Fagala and Wigg stated that selenium is an important component of glutathione peroxidase which acts to prevent the decay of cellular function, and appears to offer protection from the effects of the toxic metals lead, mercury, and cadmium (42). From our study, we can draw the conclusion that autistic children with deficient selenium become regressive and withdrawn, are unable to pay attention, show learning disorders, and are unable to play normally. These children are fussy, have tantrums provoked by the least change in their accustomed routines, such as placement of objects in the room, or time of day of events.

Our data supports the research of Pyria and Geetha which demonstrated that magnesium and selenium levels were significantly decreased ( $p < 0.001$ ) in autistic children when compared to controls (9). The significant elevation in the concentration of Cu, Pb, and Hg and the significant decrease in the concentration of Mg and Se observed in the hair and nail samples of autistic subjects well correlates with Pyria and Geetha previous findings (9).

It is believed that ASD kids have problems with the chemical pathway that allows them to detoxify metals to alleviate different cluster of autistic symptoms (9). Multiple toxic intoxication seems to affect ASD children and we could confirm significant positive correlations with a number of toxins including uranium (see Table 4).

Toxic metal exposure affects the absorption and utilization of nutrient elements. Lead replaces zinc on hem enzymes and cadmium replaces zinc on metallothionin. We noted a significant negative correlation between Zinc & Fear and Nervousness ( $p = 0.022$ ) and a significant negative correlation between Zinc & Verbal communication ( $p = 0.017$ ). Low hair zinc levels were associated with increased Fear, Nervousness and more impairment in Verbal Communication, which may enhance effect of lead, for which a significant positive correlation between lead and verbal communication ( $p = 0.020$ ) was noted (Table 4).

Zinc is affected by copper. Both trace elements, copper and zinc, are nutritive and potentially toxic, and are antagonists in function. Biochemically, a low level of zinc exasperates copper toxicity. Significantly elevated hair copper levels in the hair of autistic children are as-

sociated with neurotoxic effects, including depression, irritability, fear, nervousness, learning and behavioral disorders (43). Zinc deficiency interferes with cognitive performance (44) and the combined effect with the neurotoxic effects of lead and copper may very well be too much of a burden for the developing child.

We found a significant negative correlation between Molybdenum & Verbal communication ( $p = 0.023$ ) and General impression ( $p = 0.000$ ) (Table 5), indicating that a low molybdenum level in hair is associated with disturbed general impression and verbal communication. Copper and molybdenum are also antagonistic in function and molybdenum deficiency in the diet may be a risk factor for copper toxicity. In animal nutrition it is long known and documented that excess copper storage in the liver of sheep can be prevented by adding a few milligrams of molybdenum to their feed (45).

Our data suggests that low hair levels of molybdenum and zinc directly affect the copper and lead status and ASD symptomatology.

Lithium is a nonessential trace element, medically used to treat neurological disorders. Considered a psychiatric drug with the ability to stabilize mood disorders, lithium plays an important role in vitamin B12 transport and distribution (46). Lithium supplementations have been found to be an effective treatment in condition such as bipolar, depression, autism, and schizophrenia. Low levels of lithium have been found in children suffering from learning disabilities, incarcerated violent criminals and autism, causing abnormal brain cell balance and neurological disturbances (47). Our research data indicates (Table 5) a significant negative correlation between Lithium &

Relating to people ( $p = 0.028$ ), Emotional ( $p = 0.044$ ), Adaptation to changes ( $p = 0.027$ ), Visual response ( $p = 0.023$ ) and Total CARS ( $p = 0.013$ ). Statistically, low lithium in hair could be associated with a greater impairment in relation to people, emotion, and adaptation to changes, visual response and more total CARS.

□

## CONCLUSION

- The potentially toxic elements chromium, copper, mercury, nickel, and lead are more prevalent in the hair of children with autism as compared to age and sex matched healthy controls, correlating with ASD symptoms and Subscale and Total Score of CARS
- Nutritive elements, including calcium, iron, iodine, magnesium, manganese, zinc, and selenium are more deficient among autistic children.
- The effect of nutritional inadequacies potentially heightens the toxicity of metals.
- Biological damage from toxic material and increased environmental exposure at key times in development may play a causal role in the etiology of autistic disorders and potentially increases the severity of autistic symptoms.
- Hair analysis is of potential usefulness for the determination of toxic and essential elements in ADS children, offering an early chance for intervention and treatment.
- Further research is needed on a wider scale.

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