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Metal and essential element levels in hair and association with autism severity

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a complex disorder with heterogeneous etiology and wide clinical severity which supports the needs of recognizing biological and clinical features in patient subsets. The present study aimed to understand possible associations between the hair levels of metals and essential elements and some specific features of ASD measured by the Autism Diagnostic Observation Schedule (ADOS) that represents the gold-standard instrument to objectively confirm ASD diagnosis.

Methods: A cross-sectional study was performed in the province of Catania (Sicily, South Italy). Forty-eight subjects with ASD (70.8% male), aged from 2 to 17 years were studied. Metals (Li, Be, Al, Ni, As, Mo, Cd, Hg, U, Pb) and essential trace elements (Cr, Co, Mn, Zn, Cu, Se) were quantified in hair by inductively coupled plasma mass spectrometry analysis. Participants were characterized by measuring the severity of autism symptoms and cognitive levels.

Results: A significant and positive correlation was found between hair metal burden (lead, aluminum, arsenic and cadmium levels) and severity of ASD symptoms (social communication deficits and repetitive, restrictive behaviors). Hair zinc level were inversely related with age while there was a negative, significant association between hair zinc level and severity of autistic symptoms (defective functional play and creativity and increase of stereotyped behavior). Lead, molybdenum and manganese hair levels were inversely correlated with cognitive level (full intelligence quotient) in ASD individuals.

Conclusions: The present study suggests the importance to combine metallomics analysis with pertinent disease features in ASD to identify potential environmental risk factors on an individual level possibly in the early developmental period.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disturbance with a prevalence in Italy and in other Western countries of approximately 1% of school age children [1]. ASD is characterized by defective social interaction and communication alongside repetitive behavior, restricted interests and sensory hyper-sensitivity. The clinical spectrum is wide and it is also influenced by the severity of ASD nuclear symptoms as well as intelligence and language development [2]. ASD etiology includes mutual interactions among genetic and epigenetic backgrounds with environmental stressors such as toxicant exposure. Understanding the contribution of environmental factors to ASD is important, as these factors may be amenable to intervention, and may operate on specific inherited backgrounds. Clinical metallomics studies based on reliable inductively coupled plasma mass-spectrometry (ICP-MS) methods have been proven helpful to investigate possible disease-associated profiles [3]. In particular, essential mineral deficiency and toxic metal burden in human body estimated in hair has become a non-invasive, feasible procedure for large sample studies even in childhood [4,5]. Essential trace element levels have been associated with ASD. In particular, zinc (Zn) has been demonstrated to play a protective role against neurodevelopmental disorders including ASD due to its role in antioxidant and detoxication systems in particular the metallothionein system functioning. Plasma zinc levels may be reduced in children with ASD and associated with lowered blood zinc/serum copper ratio indicating toxic metal burden [6] Likewise, hair zinc concentration is significantly reduced in a large proportion of children with ASD, particularly in infancy [5] as well as in other neurodevelopment disorders [7].

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A number of studies explored toxic metal levels in body fluids and tissues of children with ASD compared to age-matched healthy controls (HC) with controversial results [8]. Several case-control studies reported that some parts of the children with autism have suffered from high accumulation of toxic metals such as cadmium, lead or aluminum and mercury [4]. Alongside considered study limitations including relatively small sample size, it has been noted that simply study group differences (ASD vs HC) includes the possibility that ASD clinical heterogeneity could distort the mean difference between groups when autism severity symptoms is not taken into account [8]. In this regard, only a few studies have been reported concerning the possible association between levels of toxic metals and essential elements and the severity of ASD [9–15]. Increasing evidence suggests that due to the heterogeneity of biological factors underlying ASD there is a need to stratify the ASD population according to clinical and biological markers that allow recognition of specific classes of ASD patients [16,17].

Based on the above considerations, in the present study we systematically analyzed a large panel of metals and essential elements in hair samples of children with ASD. Participants were characterized by measuring the severity of autism symptoms and cognitive levels in order to understand the possible association of hair metal and trace element levels with individual clinical features.

2. Participants and methods

We carried out a cross-sectional study in Catania province (Sicily, Italy) to investigate the relationship between hair metal/essential element levels and the severity of autism. The local Ethics Committee at the University Hospital "Policlinico-Vittorio Emanuele" in Catania approved the protocol of the present study. All procedures performed were in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments (2013). The parents of all children included in the study gave their informed written consent.

3. Study population

Caucasian subjects with ASD from various socio – economic contexts, were consecutively recruited for the study between January 2017 and December 2018 at the Child Neuropsychiatry Unit of the University Hospital "Policlinico-Vittorio Emanuele" Catania (Sicily, Italy). They were part of a larger sample of children with ASD diagnosed during the study period.

All patients were diagnosed to have an ASD according to the criteria of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association 2013) [2]. To prevent the influence of other side factors on hair trace element content, the following exclusion criteria were used: presence of other neuropsychiatric disorders including cerebral palsy (ICD-10: G80-G83) and abnormal eating behaviors (ICD-10: F50); vegetarianism; endocrine disorders (obesity, diabetes); metallic implants (including dental amalgam fillings); acute traumas and inflammatory diseases; the use of mineral-enriched shampoos and hair care products.

None of the children examined in the present study lived near industrial sources of pollution.

The study was conducted through the review of medical records of enrolled patients. Demographic and clinical data of the enrolled subjects were also collected using questionnaires. General and neurological examination and collection of hair were performed in the presence of parents (or legal representatives).

4. ASD diagnosis

Diagnosis of ASD was performed with internationally validated assessment tools, such as Autism Diagnostic Interview-Revised (ADI-R) [17] and Autism Diagnostic Observation Schedule (ADOS) [18]. The ADOS calibrated scores from the Social Affect (SA) and Restricted, Repetitive (RR) Behaviors and Play and Creativity were used as measure of autism severity in different behavioral domains. Moreover the Calibrated Severity Score (CSS) from 4 to 10 was computed to provide a continuous measure of overall ASD symptom severity [19].

5. Cognitive level measurements

Developmental quotient (DQ) and/or Intellectual quotient (IQ) were measured in all participants by a comprehensive, standardized neuropsychological assessment battery administered according to age. Specifically, children aged 2–4 years or with severe language impairment were evaluated with the Griffith Mental Development Scale GMDS [20]. The WIPPSI-III was used for verbal children aged 4–6 ½ [21] and WISC-IV was adopted for children aged 6–17 years [22]. The Leiter-R scale [23] was used for non-verbal children and adolescents aged 2–17 years. In accordance to their cognitive level, patients with IQ/DQ < 70 were considered with intellectual disability.

6. Sample collection, sample preparation and metal/essential element analysis

Only proximal parts of occipital scalp hair strands were collected using ethanol-pre-cleaned stainless steel scissors in a quantity of 1–2 g. The collected samples of hair were stored in plastic tubes labeled with patients' name, identification number and collection time and date. The samples were washed three times with double distilled water and dried at 60 °C to a stable weight. Before metal and essential elements analysis, 0.5 g of each hair sample was digested in a mixture of 6 mL of p.a. Nitric Acid (65% w/v; Merck; Darmstadt, Germany) and 2 mL of p.a. Hydrogen Peroxide (30% w/v; Sigma-Aldrich, St. Louis, USA) in acid-cleaned TFM vessels in a Microwave System (Ethos TC, Milestone; Sorisole, Italy). Digestion was carried out in three steps: heating for 15 min (power 1000 W, temperature 180 °C), digestion for 15 min (power 1000 W, temperature 180 °C) and cooling for 15 min. Blanks containing acid mixture without sample were prepared and digested in parallel. The cooled digested samples and blanks were then transferred into a 50 mL Falcon tubes and diluted with double distilled water. Metal and essential element levels in hair were determined by Inductivity Coupled Plasma – Mass Spectrometer (ICP-MS) (Elan DRC-e, Perkin Elmer, USA). System's calibration was performed using standard solutions (0.5, 5, 10, 20, 60, 80 and 100 μg/L) of trace elements prepared from commercially available Multi Elements Standard Solution (CPA-chem, accredited to ISO17034 and ISO/IEC 17025). Internal online standardization was performed using Yttrium (Y) and Rhodium (Rh) solutions (10 μg/L) prepared from Pure Single-Element Standard (CPA-chem, accredited to ISO17034 and ISO/IEC 17025). Laboratory quality control was regularly performed for each batch of mineralization with a laboratory-fortified matrix. The mean recovery rates for all analyzed metal and essential elements were within the range of 90%–110% during the whole period of investigation.

7. Statistical analysis

All statistical analysis was performed by Statistical Package for Social Sciences, version 21, software (SPSS Inc., Chicago, IL, USA). Normality of data distribution was assessed using the Kolmogorov-Smirnov test of normality. As the distribution of data was not Gaussian, descriptive statistics of hair elements content included median and interquartile interval (25th – 75th). Percentages for all nominal variables were also calculated. Mann-Whitney *U* test was used for group comparisons. The relationship between age, IQ/DQ, ADOS-CSS Calibrated SSS, ADOS behavioral domains and hair metal/essential element levels was assessed with a bivariate correlation matrix. Spearman correlation coefficients of 0.50 or greater were considered to represent a large effect size [24]. A p-value less than 0.05 ($p < 0.05$) was assumed to be statistically significant.

Table 1
Demographic data and clinical findings of the enrolled patients affected by autism spectrum disorder (N = 48).

Variables	n(%)
Gender	
Male	34 (71)
Female	14 (29)
Mean age (years) ± SD	6.5 ± 3.8
Distribuzione in classi di età	
≤ 2	6 (13)
3–7	27 (56)
8–12	12 (25)
> 13	3 (6)
Median birth weight, (kg) (IQR)	3.24 (2.91-3.64)
Perinatal medical history	
Birth asphyxia	6 (13)
Intellectual disability (ID)	31 (64.6)
Median DQ/IQ* (min-max)	56.8 (35-96)
Regression	15 (31.3)
CSS**	
ASD	6 (12.5)
AUT	42 (87.5)

* DQ: Developmental quotient/intellectual quotient.
** CSS: Calibrated Severity Score, ASD: Autism spectrum disorder; AUT: Autism.

8. Results

Table 1 shows that the mean age of children in the study was 6.5 ± 3.8 years (range: 2–17 years). More than half patients were between 3 and 7 years of age, and 70.8% was male. A positive medical history for perinatal adverse events (birth asphyxia) was reported only in 6/48 patients (13%). Intellectual Disability was diagnosed in 31/48 patients (64.6%). Regressive autism indicating loss of acquired language and social skills before the age 36 months [25] was reported in 15/48 patients (31.3%). According to CSS autism severity was graded severe in the majority of our cohort (CSS 6–10) while only 6/48 (12.5%) had less severe autism symptoms (CSS 4–5). On average, hair levels of Lithium, Aluminum, Chrome, Arsenic and Cadmium were increased whereas levels of Manganese, Zinc, Nickel and Cupper were decreased in patients with ASD compared with age-related reference values. Hair metal/essential element levels in the study cohort are reported in Table 2.

9. Correlation among clinical variables and hair mineral levels

Correlation analyses were computed between age, IQ/DQ, ADOS-CSS, ADOS behavioral domains and hair metal/essential element levels.

Table 2
Distribution of hair metal/essential element levels (μg/g).

Element	Median (IQR)
Lithium	0.025 (0.025-0.025)
Beryllium	0.025 (0.025-0.025)
Aluminum	7.738 (5.797-12.79)
Chrome	1.151 (0.714-1.714)
Cobalt	0.025 (0.025-0.025)
Manganese	0.144 (0.058-0.322)
Nickel	0.158 (0.067-0.365)
Zinc	136 (107-168)
Copper	9.226 (7.939-12.07)
Arsenic	0.082 (0.030-0.165)
Selenium	0.511 (0.465-0.610)
Molybdenum	0.117 (0.051-0.179)
Cadmium	0.021 (0.010-0.035)
Mercury	0.338 (0.121-1.255)
Uranium	0.047 (0.026-0.086)
Lead	0.542 (0.316-1.690)

Table 3
Correlation between age, cognitive level (IQ/DQ), Calibrated Severity Score (CSS) and hair metal/essential element levels.

Element	Sperman's correlation coefficient		
	Age	IQ/DQ	CSS
Lithium	−0.036	−0.110	−0.109
Beryllium	−0.143	−0.069	0.220
Aluminum	−0.244	−0.120	−0.024
Chrome	0.237	−0.109	−0.117
Cobalt	0.042	0.041	0.113
Manganese	0.081	−0.310*	0.261
Nickel	0.135	−0.100	−0.057
Zinc	0.637*	0.092	−0.048
Copper	0.175	−0.033	−0.045
Arsenic	−0.007	−0.032	−0.042
Selenium	−0.057	−0.034	−0.226
Molybdenum	−0.540*	−0.359*	0.174
Cadmium	−0.107	−0.269	0.061
Mercury	−0.138	0.063	0.041
Uranium	−0.224	−0.146	0.132
Lead	−0.445*	−0.417*	0.266

* Correlation is significant at $p < 0.05$.

In the total cohort hair levels of zinc correlated positively with age whereas a negative, significant association was found between age and hair molybdenum and lead levels (Table 3).

The relationship between the DQ/IQ, ADOS-CSS, ADOS behavioral domains and hair metal/essential element levels is outlined in Tables 3 and 4. Cognitive level measured as DQ/IQ was negatively correlated in bivariate comparisons with manganese ($rho = -.31$; n = 48; $p = .032$), molybdenum ($rho = -.36$; n = 48; $p = .012$) and lead ($rho = -.42$; n = 48; $p = .003$), and these effects were statistically significant, but weak [24]. We observed a weak correlation among hair manganese and lead levels with ADOS-CSS, a measure of overall ASD symptom severity. The correlation was not significant probably due to the small sample size (Table 3).

On the other hand, a significant positive correlation was observed when comparing hair metal levels with specific autism behavioral domains. Actually, hair levels of arsenic and cadmium significantly and positively correlated with ADOS measure of stereotyped behaviors. There was a significant, direct correlation between hair lead levels and ADOS measure indicating defective social and communication skills. The hair levels of aluminum, arsenic, cadmium and lead all correlated positively with ADOS measure indicating reduced play and creativity. Moreover, we found a significant, negative correlation between hair zinc levels and defective play as well as with increased stereotyped behavior (Table 4). Finally, there were no significant differences in the distribution of Zn, Al, As, Cd and Pb levels according to the presence of regressive autism (Table 5).

Table 4
Correlation between hair metal/essential element levels and the severity of autism.

Positive correlation
Al (rho: 0,31, p = 0,04) vs ADOS-P
As (rho: 0,31, p = 0,04) vs ADOS-P
As (rho: 0,31, p = 0,04) vs ADOS-RR
Cd (rho: 0,40, p = 0,01) vs ADOS-P e ADOS-RR (rho: 0,30, p = 0,05)
Pb (rho: 0,47, p = 0,001) vs ADOS-P
Pb (rho: 0,31, p = 0,04) vs ADOS-SA (social affect)
Negative correlation
Zn (rho: -0.39, p = 0,006) vs ADOS-P
Zn (rho: -0.32, p = 0,027) vs ADOS-SA

ADOS-P: Play and Creativity, ADOS-SA: Social Affect, ADOS-RR: Restricted Repetitive Behavior.

Table 5
Distribution of Zn, Al, As, Cd and Pb levels (μg/g) according to the presence of regressive autism.

Element	Regression		p-value*
	No N = 33	Yes N = 14	
Zn	136	130	0.762
Al	7.733	7.415	0.675
As	0.079	0.100	0.557
Cd	0.014	0.026	0.119
Pb	0.462	0.815	0.119

* Mann-Whitney *U* test.

10. Discussion

The present study aimed to understand possible associations between the hair levels of metals and essential elements and some specific features of ASD. For this purposes we considered those clinical determinants known to impact the outcome of patients with ASD, specifically the severity in social affect domain (social interaction and communication) as well as the intensity of restricted interests and stereotyped behavior measured by the Autism Diagnostic Observation Schedule that represents the gold-standard instrument to objectively confirm ASD diagnosis using standard criteria. We also considered the individual cognitive level, as intellectual disability is the most common neurological comorbidity of ASD (50–60%) and an important predictive factor for the outcome of patients with ASD [25,26].

We found a significant inverse correlation of toxic metals such as lead and molybdenum levels in hair with age ($p < 0.001$) suggesting that among patients with ASD, younger children might be more vulnerable to toxic metal burden than older individuals. Moreover, we found that lead, molybdenum as well as manganese levels measured in hair were inversely correlated ($p < 0.05$) with the cognitive level in the study group supporting a possible role of toxic metal burden in patients with ASD and low intellectual functioning. In the same line, the present study demonstrated a significant and positive correlation between hair metal burden (lead, aluminum, arsenic and cadmium levels) and severity of ASD symptoms measured by ADOS. In particular, our findings suggest a correlation between severity of social communication deficits and repetitive, restrictive behaviors that are core features of ASD with toxic metal burden measured in hair. A previous study found a significant association between toxic metal levels in blood, increased oxidative stress and reduced glutathione level and autism severity with the most consistent results for mercury and cadmium [27].

One other study reported that higher scores on the CARS (indicating more severe autism symptoms) were significantly related to higher hair and nail mercury and lead levels [28]. Likewise, higher lead levels in hair [29] and in blood [30] were correlated with lower intellectual functioning in children with ASD. In line with the present study, higher hair levels of lead were consistently correlated with verbal communication problems and more ASD symptoms measured by CARS [31]. Such observations prompted to analyze the effects of treatments by detoxification methods in ameliorating biomarkers of toxicants [9,32] or clinical symptoms in children with ASD [33]. No adverse effects were reported in these studies however; additional studies including control groups and placebo-controlled studies are warranted to confirm possible beneficial effects of these treatments.

Putative mechanisms underlying the increase of toxic metal levels in patients with ASD include modification in exposition, absorption and excretion such as reduced levels of glutathione (which binds to toxic metals and excretes them in the bile) and excessive oral antibiotic use (which greatly inhibits fecal excretion) as well as polymorphisms in genes associated with increased susceptibilities to environmental toxicants [8,9].

Some toxic elements such as cadmium, arsenic and mercury, and also essential mineral deficiencies may induce epigenetic alterations and play a possible role in neurodevelopmental disorders. Epigenetic changes driven by environmental stressors may negatively influence biological pathways important for ASD development [34]. Methylation changes have been found in several ASD candidate genes [34]. More recently, aberrations in histone acetylation were demonstrated in the prefrontal and temporal cortex of patients with ASD [35]. Epigenetic changes have been associated with genes related to ion channels, synaptic function, and epilepsy/neuronal excitability, all of which have previously been shown to be dysregulated in ASD [34,35]. In the present study we show that hair zinc level are inversely related with age in children with ASD while there is a negative, significant association between hair zinc level and severity of autistic symptoms (defective functional play and creativity and increase of stereotyped behavior). Interestingly, a strong association between defective hair zinc level and ASD has been proven in a population study including 1967 children with ASD showing that 43.5% of younger children (age 0–3 year-old) suffered from marginal to severe zinc deficiency [5]. In addition to ASD [36] low serum zinc levels have been reported in children with other neurodevelopmental disorders such as Attention Deficit Hyperactivity Disorder (ADHD) [37] pointing to a causative role between zinc deficiency and proper nervous system development and function [38]. Systemic zinc deficiency as assessed in hair or blood samples appears to reflect also brain tissue, with decreased Zn concentrations in hippocampus and cerebral cortex accompanied by reduced learning behavior in a rat model of diet-induced Zn deficiency [39]. Some candidate genes such as ZnT5 (zinc transporter 5), ERK1 (extracellular signal-regulated kinase 1), TrkB (tyrosine-related kinase B), SHANK2, and SHANK3 associated with ASD development, are influenced by Zn and/or play a role in Zn-singnaling and metal ion homeostasis thus providing an explanatory mechanism of gene/environment interaction [40].

In conclusion we show a significant, positive association between severity of autism behavioral domains measured by standardized measures and lower cognitive functioning with toxic metal levels in hair of children with ASD. Moreover we underline that decreased hair zinc levels are associated with ASD and in particular with more severe autism symptoms. Limitations of the present study include a relatively small sample size. Furthermore, the mechanisms responsible for imbalances of metals and essential elements in this subset of ASD patients cannot be inferred from our investigation. Further research is required to unravel molecular mechanisms underlying mineral imbalances and severity of autism symptoms. As a whole, the present study strongly suggests the importance to combine clinical metallomics with pertinent disease features in patients with ASD to identify putative environmental risk factors on an individual level, possibly in the early developmental period.

Declaration of Competing Interest

The authors declare no conflict of interest.

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