Associations of heavy metal levels with environmental and behavioral factors in children with autism spectrum disorder

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Abstract

Background

The etiologies of autism spectrum disorder (ASD) are yet unclear. Previous studies suggested that ASD is associated with environmental heavy metals. Thus, the present study analyzed the levels of 41 heavy metals and the associated environmental factors in children with ASD.

Methods

The 25 children diagnosed with ASD were included in the case group (ASD group), while the 18 age- and gender-matched healthy children who came for routine care were included in the typical development group (TD group). The levels of heavy metal in the blood were measured in both groups. The questionnaire survey collected the demographic information, socioeconomic information, and risk factors of potential heavy metal sources for the analysis of risk factors.

Results

A total of 25 children were included in the ASD group and 18 in the TD group. The blood manganese (Mn) level was significantly higher in the ASD group than the TD group . The father's educational level was significantly higher in the ASD group than the TD group. The living status was mainly scattered for the ASD children and daycare for the TD group. The frequency of book-reading, washing hand with sanitizer/soap, and the folic acid intake by the mother before pregnancy was significantly lower in the ASD group than the TD group, while the percentage of birth disorder history was significantly higher in the ASD group than the TD group. Binary logistic regression analysis showed that the folic acid intake by the mother before pregnancy and father's education level beyond junior college were protective factors for ASD. Also, the frequencies of washing hands with sanitizer every time, sometimes, and hardly acted as protective factors for ASD.

Conclusion

Blood Mn level was significantly higher in ASD than TD, suggesting that environmental Mn exposure could be a risk factor of ASD in children. Folic acid intake by the mother before pregnancy and father's education levels are protective factors for ASD. Concentrated heavy metal in the blood in prenatal or early life exposures which suggested ASD is needed in the future study.

Background

Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder with the incidence increased sharply in recent 15 years. A survey released by the Centers For Disease Control And Prevention (CDC) on April 27, 2018 has caused concern that the prevalence of autism is as high as 1/59, a 16% increase over the 1/68 data volume published on the CDC on March 27, 2014, entitled "CDC estimates 1 in 68 children has been identified with autism spectrum disorder.",and a 181% increase over the 1/166 data volume published on the CDC in 2004.The rehabilitation training for ASD patients economically burdens families and society. Thus, it is imperative to investigate the etiologies of ASD. Hitherto, studies have demonstrated that increased levels of lead (Pb), mercury(Hg), and arsenic(As) in the air are associated with an increased prevalence of ASD [1]. In addition, data from the center for disease control and prevention of the USA have demonstrated the associations of environmental levels of Pb, Hg, and As with ASD [2].

Interestingly, the levels of heavy metal in blood, urine, teeth, hair, and related body fluid or tissues in ASD children are inconsistent. Some studies demonstrated that the levels of Pb, Hg, As, Cu, and Cd were significantly higher in the autism children than healthy controls [3], while the significantly reduced levels of Mg and Se were well associated with the severity of ASD [4]. Some studies even used chelating agents to reduce the blood levels of Pb and Hg and found that the symptoms of autism alleviated with the reduced blood Pb and Hg levels [5, 6]. Animal studies also showed an association between Pb level and autism symptoms [7, 8]. However, some studies proposed that after controlling the potential confounding factors, the geometric mean value of the blood Pb level was not significantly different between the ASD patients and controls [9]. In addition, the excretion rate of Pb was not significantly affected in ASD children, and the urine Pb level was not associated with any core symptoms in ASD children [10].

Currently, only some heavy metals have been investigated in ASD children. In this study, 41 heavy metals were measured in ASD and healthy children. To the best of our knowledge, this is the first study with the most types of heavy metals measured in ASD children. In addition, we also investigated the influencing factors of the sources of heavy metals, which laid the foundation for the investigation of the correlations between ASD and heavy metals in children.

Methods

Subjects

A total of 25 children diagnosed with ASD in the Outpatient Department of Child Healthcare in the Guangdong Women’s and Children’s Hospital between September and November 2018 were included in the ASD group. These children were diagnosed independently by two physicians, according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition, DSM-5) (American Psychiatric Association, 2013). In addition, 18 age- and gender-matched children that visited the hospital for routine care were included in the typical development group (TD). The children who were diagnosed with central nervous system diseases, organic diseases, or inherited metabolic diseases after birth were excluded.

Estimation of heavy metals

A volume of 100 μL of blood was mixed with 200 μL of 50% (v/v) hydrogen nitrate and 30 μL hydrogen peroxide, followed by heating in a water bath at 90 °C for 3 h until transparent. After cooling to room temperature, the blood samples were diluted to 2.5 mL with ultrapure water. The concentration of metals in the blood was measured by inductively coupled plasma mass spectrometry 8800 (Agilent, Palo Alto, CA, USA). Seven element standards were used for ICP-MS calibration, including 8500–6940 “multi-element calibration standard 2A” (Agilent); GSB 04-1769-2004 “multi-element calibration standard” (Guobiao (Beijing) Testing & Certification Co., Beijing, China); GSB 04-1789-2004, “multi-element calibration standard” (Guobiao Testing & Certification Co.); GSB 04-1767-2004, “multi-element calibration standard” (Guobiao Testing & Certification Co.); GSB 04-1727-2004, “single-element calibration standard” (Guobiao Testing & Certification Co.); GSB 04-1760-2004, “single-element calibration standard” (Guobiao Testing & Certification Co.); 8500 – 6940, “multi-element calibration standard 2A” (Agilent).

Behavioral assessment and environmental survey

A questionnaire survey was conducted by uniformly trained investigators to collect data of the children in the ASD and TD groups with respect to demographics, life environment, lifestyle, pregnancy, and delivery. Informed consent was obtained from the patients for the questionnaire survey. The questionnaire data were double-input by Epidata software.

Statistical analysis

SPSS 17.0 software was used for statistical analysis. The levels of heavy metals in normal distribution were analyzed by independent t-test in the ASD and TD groups, while for those not in a normal distribution, the non-parametric U test was used for the analysis in the two groups. Chi-square test was used for the comparison of demographic data, life environment data, lifestyle, and information of the maternal pregnancy and delivery between the two groups. Binary logistic regression was used to investigate the influencing factors of ASD in children. $p < 0.05$ was considered statistically significant.

Results

General description

A chi-square test was used for the comparison of the demographic data of the children between the ASD and TD groups. For 25 children in the ASD group (21 boys and 4 girls) and 18 children in the TD group (13 boys and 5 girls), the distribution of the genders did not differ significantly (χ^2=-0.877, p = 0.349). In addition, the age of the children in the ASD and TD groups (3.48 ± 1.534 vs. 3.78 ± 1.762) years did not differ significantly (t=-0.606, p = 0.548). More ASD children were found in other areas than TD children (64.0% vs. 33.3%, χ^2 = 3.94, p = 0.047). The proportion of the father’s educational level more than junior college was significantly lower in the ASD group than the TD group (40.0% vs. 83.3%, χ^2 = 8.18, p = 0.017). The living status of the ASD children was mainly scattered (68.4%), while the TD children were primarily in daycare (70.6%) (χ^2 = 5.495, p = 0.019), and the difference between the two groups was statistically significant. The parents’ ages at delivery and the mother’s educational level were not significantly different between the two groups (Table 1).

Table 1
Comparison of demographic and socioeconomic data between the ASD and TD groups

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
Gender	Male	21 (84.0)	13 (72.2)	-0.88	0.35
Age (years)	1–2	12 (48.0)	8 (44.4)	0.94	0.63
	3–4	9 (36.0)	5 (27.8)		
	5–8	4 (4.0)	5 (27.8)		
Family address	Guangzhou	9 (36.0)	12 (66.7)	3.94	0.047
	Other areas	16 (64.0)	6 (33.3)		
Maternal age at the childbirth [1]	< 35 years	21 (87.5)	14 (77.8)	0.7	0.44
	≥ 35 years	3 (12.5)	4 (22.2)		
Paternal age at the childbirth [2]	< 35 years	19 (79.2)	13 (72.2)	0.27	0.72
	≥ 35 years	5 (20.8)	5 (27.8)		
Mother's educational level	≥Junior college	10 (40.0)	13 (72.2)	4.573	0.102
	High school/professional high school/vocational school/technical secondary school	8 (32.0)	2 (11.1)		
	≤Junior high school	7 (28.0)	3 (16.7)		
Father's educational level	≥Junior college	10 (40.0)	15 (83.3)	8.18	0.017
	High school/professional high school/vocational school/technical secondary school	8 (32.0)	2 (11.1)		
	≤Junior high school	7 (28.0)	1 (5.6)		

1, 1 missing data of the "maternal age at the childbirth" in the ASD group

2, 1 missing data of the "paternal age at the childbirth" in the ASD group

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
Living status	Scattered	16 (68.4)	5 (23.5)	5.495	0.019
	Daycare	9 (31.6)	13 (70.6)		
	Boarding nursery	0 (0.0)	0 (0.0)		
1, 1 missing data of the “maternal age at the childbirth” in the ASD group					
2, 1 missing data of the “paternal age at the childbirth” in the ASD group					

Assessment of levels of heavy metals

A total of 41 heavy metals were detected in the blood of the children in the two groups. While 18 could be detected, the levels of the other 23 heavy metals were 0 between the ASD and TD groups. The blood manganese (Mn) level was significantly higher in the ASD group than the TD group (43.33 ± 14.79 v.s 35.05 ± 10.59, t = 2.028, p = 0.049), while that of the other heavy metals did not differ significantly between the two groups (Appendix Tables 1–2).

Table 2

Comparison of influencing environmental factors between the ASD and TD groups

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
Smoking of the cohabitant	Yes	13 (52.0)	9 (50.0)	0.17	0.897
Number of cigarettes the cohabitant smoked (daily) [1]	< 5	4 (30.7)	7 (77.8)	3.98	0.263
	5–10	5 (38.5)	2 (22.2)		
	> 10	2 (15.4)	0 (0.0)		
Heavy metal-associated job of the father	1 = yes, 0 = no	3 (12.0)	1 (5.6)	0.515	0.438
Heavy metal-associated job of the mother	1 = yes, 0 = no	1 (4.0)	1 (5.6)	0.057	0.668
Floor of the apartment they currently live in [2]	One-storey house/ground floor	2 (8.0)	1 (5.6)	0.71	0.871
	2–3 floors	9 (36.0)	5 (27.8)		
	4–6 floors	6 (24.0)	6 (33.3)		
	≥ 7 floor	7 (28.0)	6 (33.3)		
Distance from the apartment to the heavy-loaded main road	Storefront	7 (28.0)	4 (22.2)	7.129	0.124
	> 7 m (separated by greenbelt)	2 (8.0)	6 (33.3)		
	> 15 m (separated by one building)	5 (20.0)	1 (5.6)		
	> 30 m (separated by two buildings)	3 (12.0)	2 (11.1)		
	Distant from main road	8 (32.0)	5 (27.8)		
Usage of aluminum pot	Yes	2 (8.0)	3 (16.7)	0.765	0.382

1, 2 missing data of the “number of cigarettes the cohabitant smoked” in the ASD group

2, 1 missing data of the “floor of the apartment they currently live in” in the ASD group

3, 2 missing data of the “Paint peel off from the apartment walls or furniture” in the ASD group

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
Type of fuel used in the kitchen	Coal gas	7 (28.0)	5 (27.8)	4.050	0.256
	Natural gas	10 (40.0)	3 (16.7)		
	Coal/firewood/grass/coal products/others	8 (32.0)	10 (55.6)		
Existence of factors around the apartment	Yes	9 (36.0)	3 (16.7)	2.77	0.096
Decoration of the department in the recent 2 years	Yes	4 (16.0)	5 (27.8)	0.523	0.470
Painting peel off from the apartment walls or furniture [3]	Yes	3 (12.0)	3 (16.7)	0.106	0.745
Walking time of the child on the main road (h)	Hardly	9 (36.0)	9 (50.0)	5.071	0.651
	0–1 h	6 (24.0)	5 (27.8)		
	1–2 h	6 (24.0)	2 (11.1)		
	≥ 3 h	4 (16.0)	2 (11.1)		
Type of the favorite toys	Plastic toys	23 (92.0)	12 (66.7)	5.12	0.077
	Others	2 (8.0)	6 (33.3)		
Times of washing the toys	Almost every day	4 (16.0)	3 (16.7)	2.529	0.470
	1–2 times per week	7 (28.0)	9 (50.0)		
	1–2 times per month	7 (28.0)	3 (16.7)		
	< 2 times per year	7 (28.0)	3 (16.7)		
Frequency of book-reading	Almost every day	5 (20.0)	13 (72.2)	12.275	0.006
	1–2 times per week	10 (40.0)	2 (11.1)		

1, 2 missing data of the “number of cigarettes the cohabitant smoked” in the ASD group
2, 1 missing data of the “floor of the apartment they currently live in” in the ASD group
3, 2 missing data of the “Paint peel off from the apartment walls or furniture” in the ASD group

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
	1–2 times per month	5 (20.0)	2 (11.1)		
	< 2 times per year	5 (20.0)	1 (5.6)		
Washing hands before meals	Hardly	2 (8.0)	0 (0.0)	2.596	0.273
	Sometimes	10 (40.0)	5 (27.8)		
	Almost every time	13 (52.0)	13 (72.2)		
Washing hands with sanitizer/soap	Every time	2 (8.0)	8 (44.4)	15.009	0.001
	Sometimes	9 (36.0)	9 (50.0)		
	Hardly	14 (56.0)	1 (5.6)		
1, 2 missing data of the “number of cigarettes the cohabitant smoked” in the ASD group					
2, 1 missing data of the “floor of the apartment they currently live in” in the ASD group					
3, 2 missing data of the “Paint peel off from the apartment walls or furniture” in the ASD group					

Environmental and behavioral factors

A chi-square test was used to compare the influencing environmental and behavioral factors between the ASD and TD groups. The frequency of book-reading and washing hands with sanitizer was significantly lower in the ASD group than the TD group (20.0% vs. 72.2%, χ^2 = 12.275, p = 0.006; 8.0% vs. 44.4%, χ^2 = 15.009, p = 0.001, respectively). However, the other factors did not differ significantly between the two groups (Table 2).

Influencing factors in pregnancy and delivery

A chi-square test was adopted to compare the influencing factors in pregnancy and delivery between children in the ASD and TD groups. Intriguingly, the frequency of folic acid intake by the mother before pregnancy was significantly lower in the ASD group than the TD group (39.1% vs. 75.0%; χ^2 = 4.855, p = 0.027), while the history of birth disorders was significantly higher in the ASD group than TD group (32.0 vs. 5.6%,χ^2 = 4.422, p = 0.035). However, no statistically significant differences were detected in the other factors between the two groups (Table 3).

Table 3
Comparison of the influencing factors in pregnancy and delivery between the ASD and TD groups

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
Gravidity	3	16 (64.0)	10 (55.6)	0.962	0.618
	2	8 (32.0)	7 (38.9)		
	1	1 (4.0)	1 (5.5)		
Preterm birth	Yes	1 (5.0)	3 (25.0)	1.990	0.158
Delivery mode [1]	Spontaneous delivery	19 (82.6)	9 (64.3)	2.745	0.254
	Cesarean section	3 (13.0)	2 (11.1)		
	Forceps delivery	1 (4.4)	3 (16.7)		
Intake of folic acid before pregnancy by the mother [2]	Yes	9 (39.1)	12 (75.0)	4.885	0.027
Duration of folic acid intake before pregnancy [3]	1	4 (50.0)	6 (54.5)	0.130	0.973
	2	2 (25.0)	2 (18.1)		
	3	2 (25.0)	3 (27.2)		
Intake of folic acid by the mother during pregnancy	Yes	22 (88.0)	14 (77.8)	0.802	0.314
Frequency of fish-eating by the mother within 3 months before pregnancy	5–7 times per week	3 (12.0)	4 (22.2)	0.998	0.804
	3–4 times per week	4 (16.0)	2 (11.1)		

1, 2, and 4 missing data in the ASD and TD groups, respectively
2, 2, and 2 missing data in the ASD and TD groups, respectively
3, 1, and 1 missing data in the ASD and TD groups, respectively
4, 4 and 2 missing data in the ASD and TD groups, respectively;
5, 6 and 4 missing data in the ASD and TD groups, respectively.

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
	1–2 times per week	11 (44.0)	8 (50.0)		
	1–2 times per month	7 (28.0)	4 (22.2)		
	Hardly	0 (4.0)	0 (0.0)		
Frequency of fish-eating by the mother during the pregnancy [4]	5–7 times per week	3 (14.3)	2 (12.5)	1.029	0.905
	3–4 times per week	3 (14.3)	4 (25.0)		
	1–2 times per week	9 (42.9.0)	5 (31.25)		
	1–2 times per month	3 (14.3)	3 (18.75)		
	Hardly	3 (14.3)	2 (12.5)		
Breast-feeding [5]	Yes	18 (94.7)	11 (78.6)	1.977	0.193
Frequency of fish-eating by the mother during breast-feeding	5–7 times per week	2 (11.1)	3 (27.3)	7.222	0.125
	3–4 times per week	3 (16.7)	2 (18.2)		
	1–2 times per week	6 (33.3)	5(45.5)		
	1–2 times per month	7 (38.9)	0 (0.0)		
	Hardly	0 (0.0)	1(9.0)		
Disease history of the child	Yes	8 (32.0)	1 (5.6)	4.422	0.035
Family history	Yes	5 (20.0)	3 (16.7)	0.121	0.941
1, 2, and 4 missing data in the ASD and TD groups, respectively					
2, 2, and 2 missing data in the ASD and TD groups, respectively					
3, 1, and 1 missing data in the ASD and TD groups, respectively					
4, 4 and 2 missing data in the ASD and TD groups, respectively;					
5, 6 and 4 missing data in the ASD and TD groups, respectively.					

Influencing dietary and behavioral factors

Chi-square test was adopted to compare the influencing dietary and behavioral factors between the ASD and TD groups, which showed that partial eclipse and learning difficulty were significantly different between the two groups. The frequency of partial eclipse and learning difficulty was significantly higher in the ASD group than the TD group (72.1% vs. 38.9%, χ^2 = 4.714 l, p = 0.030; 68.0% vs. 11.1%; χ^2 = 13.733, p = 0.001, respectively). No statistically significant differences were observed in the other factors between the two groups (Table 4).

Table 4
Comparison of the dietary and behavioral factors between the ASD and TD groups

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
Eating/drinking animal milk or corresponding products everyday	Almost every day	11 (44.0)	9 (50.0)	0.551	0.907
	1–2 times per week	3 (12.0)	3 (16.7)		
	1–2 times per month	2 (8.0)	1 (5.6)		
	Hardly	9 (36.0)	5 (27.8)		
Eating canned food/drinking	Almost every day	0 (0.0)	0 (0.0)	5.021	0.081
	1–2 times per week	4 (16.0)	0 (0.0)		
	1–2 times per month	2 (8.0)	0 (0.0)		
	Hardly	19 (76.0)	18 (100.0)		
Eating preserved eggs	Almost every day	1 (4.0)	0 (0.0)	2.322	0.508
	1–2 times per week	1 (4.0)	0 (0.0)		
	1–2 times per month	1 (4.0)	0 (0.0)		
	Hardly	22 (88.0)	18 (100.0)		
Eating puffed food	Almost every day	0 (0.0)	0 (0.0)	3.555	0.169
	1–2 times per week	3 (12.0)	0 (0.0)		
	1–2 times per month	4 (16.0)	6 (33.3)		
	Hardly	18 (72.0)	12 (66.7)		
Intake of calcium, zinc, or iron supplements (one or more types)	Almost every day	4 (16.0)	6 (33.3)	2.403	0.493
	1–2 times per week	3 (12.0)	3 (16.7)		

Parameter	Classification	ASD group (n = 25) N (%)	TD group (n = 18) N (%)	χ^2	p
	1–2 times per month	5 (20.0)	3 (16.7)		
	Hardly	13 (52.0)	6 (33.3)		
Always sucking fingers	Yes	7 (28.0)	5 (27.8)	0.000	0.987
Always biting nails	Yes	3 (12.0)	2 (11.1)	0.008	0.929
Sleep little and night terror	Yes	3 (12.0)	4 (22.2)	0.802	0.307
Anorexia	Yes	9 (36.0)	2 (11.1)	3.405	0.065
Partial eclipse	Yes	18 (72.0)	7 (38.9)	4.714	0.030
Allotriophagia	Yes	3 (12.0)	1 (5.6)	0.515	0.473
Frequent abdominal pain	Yes	1 (4.0)	3 (16.7)	1.990	0.158
Constipation	Yes	8 (32.0)	2 (11.1)	2.559	0.110
Always biting pencils	Yes	3 (12.0)	1 (5.6)	0.515	0.473
Learning difficulty	Yes	17 (68.0)	2 (11.1)	13.733	0.001
Offensive and destructive	Yes	7 (28.0)	5 (27.8)	0.000	0.987
Tic disorders	Yes	2 (8.0)	0 (0.0)	1.510	0.219

Logistic regression analysis

The factors with statistically significant differences between the ASD and TD groups were included in the logistic regression model, which encompassed the living status (scattered, daycare, and boarding nursery), family address (Guangzhou or other areas), father's educational level (≥ or < junior college), frequency of book-reading (1, every day; 2, 1–2 times per week; 3, 1–2 times per month; and 4, < 2 times per year), frequency of washing hands with sanitizer (hardly, sometimes, and every time), and intake of folic acid by the mother during pregnancy (yes or no). The binary logistic regression was conducted with the stepwise forward mode, and the model obtained is shown in Table 5 − 1. Taken together, the intake of folic acid by the mother before pregnancy (B=-2.425, p = 0.05) and father's educational level ≥ junior college (B=-3.133, p = 0.014) were protective factors for ASD. In addition, comparing with the frequency of washing hands with sanitizer every time, sometimes (B=-4.375, p = 0.012) and hardly (B=-2.960, p = 0.043) also served as the protective factors for ASD (Tables 5 and 6).

Table 5
Logistic regression of the influencing factors of ASD

-2log likelihood	Cox & Snell R^2	Nagelkerke R^2	χ^2	p
25.283	0.506	0.682	27.519	< 0.001

Table 6
Logistic regression analysis of the influencing factors in children

	B	SE	Wald	df	p	Exp (B)	95% CI	
							Lower	Upper
Intake of folic acid by the mother before pregnancy (1: Yes; 0: No)	-2.425	1.237	3.842	1	0.05	0.089	0.008	1.00
Frequency of washing hands with soap or sanitizer (1)*			6.436	2	0.04			
Frequency of washing hands with soap or sanitizer (2)	-4.357	1.731	6.336	1	0.012	0.013	0.000	0.381
Frequency of washing hands with soap or sanitizer (3)	-2.960	1.461	4.106	1	0.043	0.052	0.003	0.907
Father’s education level#	-3.133	1.269	6.09	1	0.014	0.044	0.004	0.525
Constant	6.092	2.156	7.987	1	0.005	442.234		

* Frequency of washing hands with soap or sanitizer: (1) every time; (2) sometimes; (3) hardly

#father’s education level: 1 ≥ junior college; 0 < junior college

Discussion

In this study, the levels of heavy metals in the blood were measured in the children in ASD and TD groups, which showed that the blood Mn level was significantly higher in the ASD group than the TD group. However, no significant differences were detected in the levels of other 40 heavy metals, including Pb and Hg, between the two groups in this study.

To date, the findings of the levels of heavy metals in ASD children are inconsistent. Some studies have reported that the Pb, Hg, As, and Al levels are significantly higher in ASD children than TD children. A study in Egypt measured the Hg, Pb, and Al levels from the hair of 100 ASD children and 100 TD children, which showed that the levels of Hg, Pb, and Al were significantly higher in the ASD group than the TD group. In addition, the levels of Hg, Pb, and Al were positively associated with the consumption of fish by the mother, living near the gas station, and usage of aluminum plates [11]. However, the levels of heavy metals were measured in the hair, and thus the results could only reflect the previous exposures. Another study compared the levels of heavy metals in the hair and fingernails between ASD and control groups, which showed that the levels of toxic metals, including Pb and Hg, were significantly higher in the ASD children than the control group ($p < 0.001$), while those of trace elements, including Mg and Se, were significantly lower in the ASD children than control group ($p < 0.001$). Another study also demonstrated that the degree of ASD in the children was significantly associated with the Cu levels in the hair and fingernails ($p < 0.001$) [4]. Significantly increased Cu, Pb, and Hg levels, as well as significantly decreased Mg and Se levels in the hair and fingernails were found in the ASD children and were markedly associated with the severity of ASD [4].

The findings of this study showed that the blood Mn level was a risk factor of ASD, while the other heavy metals, including Pb, Hg, and As, were not associated with an increased risk of ASD. To date, only a few studies have investigated the association between Mn and ASD, while the majority of the studies focused on the associations between other heavy metals, such as Pb, Hg, and As, and ASD. In addition, some studies demonstrated that if the mother eats fish, the prenatal Hg level in full-blood does not exert a significant influence on autism or autistic characteristics [12]. The study also showed that the blood Hg level was not significantly different between the ASD and TD groups. Another study showed that Cd level was significantly higher in the children with passive smoking than the non-passive smoking children, while Hg and Pb levels were positively associated with the consumption of seafood and body mass index (BMI) [4]. The findings of the current study showed that the blood Mn level was a risk factor of ASD, suggesting its role in the pathogenesis of ASD, especially in the studies investigating the early maternal exposure and fetal exposure, which could provide a deep insight into preventing ASD.

In some other studies, chelating agents were used to significantly reduce the blood Pb and Hg levels, which were mirrored by the alleviated symptoms of autism [5, 6]. Heavy metal levels, such as Cr, Cd, and Pb, were assessed in the urine in autist and healthy children. The findings demonstrated that compared to healthy children, the urine levels of Cd and Pb were significantly lower in the ASD children ($p < 0.05$). On the other hand, the urine Cr level was significantly higher in the ASD children than the healthy controls ($p < 0.05$). These findings indicated that ASD could be associated with the significantly reduced excretion rate of Cd(ASD&TD = 0.45 ± 0.32&1.43 ± 1.16, $p < 0.001$) and Pb（ASD&TD = 1.19 ± 1.98&4.63 ± 3.83, $p < 0.001$）, while the excretion rate of Cr（ASD&TD = 26.40 ± 16.07&11.27 ± 5.04, $p < 0.001$）in urine was significantly increased [13]. Also, the levels of Hg, Pb, and Zn were measured in the teeth of ASD and healthy children, which showed that the teeth Hg level was significantly higher in the ASD children (2.1-fold) than the controls, while the Pb and Zn levels were similar between the two groups [3]. Furthermore, the Pb (31.9 μg/L in ASD children, and 18.6 μg/L in healthy children), Hg (3.83 μg/L in ASD children and 1.09 μg/L in healthy children), and Cd (0.70 μg/L in ASD children, and 0.26 μg/L in healthy children) levels were significantly higher in ASD than healthy controls ($p < 0.01$, 0.05). In addition, the levels of essential elements, including Zn (ASD 4552.0 μg/L in ASD children, and 5118.6 μg/L in healthy children), Se (61.7 μg/L in ASD children, and 90.6 μg/L in healthy children), Mn (13.5 μg/L in ASD children, and 21.4 μg/L in healthy children) were significantly lower in ASD children than the controls [14].

A previous study in Jamaica used a univariate general linear model and demonstrated that the geometric mean value of blood Pb level was significantly different between ASD patients and controls (2.25 μg/dL in ASD patients and 2.73 μg/dL in controls, $p < 0.05$). However, the geometric mean value of the blood Pb level was not significantly different between the two groups (2.55 μg/dL vs. 2.72 μg/dL, $p = 0.64$) after the potential confounding factors were adjusted [9]. A Spanish study demonstrated that the excretion rate of Pb is not significantly affected in ASD children, and the urine Pb level was not associated with any core symptoms in ASD children [10]. Moreover, the Al level in the brain tissues of ASD children was higher than in healthy controls [15], which was inconsistent with the findings of the present study, which did not detect any significant difference in the Al level between ASD children and controls. Nonetheless, the differences might be attributed to the diversified regions of residence of the subjects, as the levels of heavy metals in the soils could vary greatly. In addition, other confounding factors, such as dietary habits, could also affect the results. Furthermore, the various tissues used for the measurement of heavy metals across the studies could also be one of the causes of the differences in the findings.

In this study, no statistically significant differences were detected in Pb and Hg levels between the ASD and TD groups; however, the Mn level was significantly higher in the ASD group than the TD group. Mn is required for the developing brain, and therefore, the transplacental absorption is maximized during pregnancy [16]. However, an increasing variety of natural and anthropogenic sources of Mn in the environment increases the pre- and postnatal early-life exposure, which indicates the association with the behavior of children/adolescents [17]. The absorbed Mn is conveyed in the blood bound to proteins (transferrin, alpha-2-macroglobulin) and in 85% of the red blood cells. The brain has a small proportion of Mn deposit, albeit with prolonged retention times. Compared to the blood Mn levels between ASD children and healthy Jamaican children, Rahbar et al. did not find any significant association between BMn and ASD, suggesting a lack of significant association between Mn exposures and ASD [9]. However, several studies examined the correlation between ASD and Mn exposure, as measured based on air distribution, tooth enamel, hair, urine, and red blood cells, but with conflicting findings. Rossignol et al. provided evidence of the association between environmental toxicants and ASD [18]. The study also highlighted an Mn study conducted with 325 children with ASD vs. 22,101 controls with respect to the perinatal exposure to the highest vs. lowest quintile of air pollutants that were significantly associated with an increased risk of ASD, including Mn (OR = 1.5; 95%CI, 1.1–2.2).

Differences in techniques for sampling and assessment of levels of heavy metals may influence the results. The teeth of the fetus are optimal samples for the assessment of cumulative exposure to toxic metals during fetal development and early infancy; however, the samples could not be obtained easily. Hair and fingernail reflect the recent cumulative exposure to heavy metals, but the color and position of the hair, as well as external contaminations, could affect the results. Conversely, blood levels reflect the cumulative levels recently. Considering that the portion of Mn excreted in the urine is as low as about 6% of the total, urine Mn is not a feasible indicator. Therefore, blood Mn could be used to assess exposure during a specific period, and investigating the differences in heavy metals in ASD children could provide further evidence and clues.

In this study, binary logistic regression analysis was conducted on the influencing factors with statistically significant differences between the ASD and TD groups. The results showed that the father's educational level, frequency of washing hand with sanitizer/soap, and intake of folic acid by the mother before pregnancy were included in the model. However, other factors, including family address, living status, frequency of book-reading, and history of birth disorders, were not included in the model. These findings demonstrated that the frequency of folic acid intake by the mother before pregnancy was significantly lower in the ASD group than the TD group. Moreover, previous studies demonstrated that supplementation of folic acid by the mother could help reduce the ASD risk of offspring, which was in agreement with the findings in this study [19]. However, over-supplementation of folic acid must also be avoided, as it has also been demonstrated to be a risk factor of ASD [20]. Furthermore, a large number of ASD patients had birth disorders than TD children. Some previous studies also demonstrated that preterm birth is a high-risk factor of ASD [21]. However, this factor was not included in the regression model, which could be due to the relatively small sample size. Thus, additional studies with larger sample sizes are needed to verify the current findings.

Limitations

Considering that the best assessment of Mn exposure is not yet well-established, we applied blood manganese concentrations as our exposure assessment. However, the bone and teeth tissue may be optimal indicators of prenatal and perinatal exposures. Several studies indicated that the Mn levels of hair increase over years of exposure partially influenced by external exposures [22]. Therefore, we used blood Mn concentration, which is an acceptable biomarker. A correlation was established between blood Mn concentrations and ASD, further affirming that the sample size needs to be increased for confirmation. Also, concentrated heavy metal in the blood in prenatal or early life exposures was lacking, which suggested ASD.

Conclusions

The etiology of ASD is not well-understood, especially with respect to mental factors. However, this study firstly measured 41 heavy metals in the children in ASD and TD groups, which expanded the range of heavy metals measured and comprehensively screened the heavy metals that might affect ASD. In addition, this study also adjusted various influencing factors for ASD, including the demographics, environmental, pregnancy, and delivery data, which could control the confounding factors. The findings of this study demonstrated that the blood Mn level was a risk factor of ASD. Thus, in future studies, the sample sizes would be increased for further analysis, and the early maternal and fetal exposures would be to elucidate the etiologies of ASD.

Declarations

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JW, JC,HH and YH conceived the project. JW, JC,HH, BC and LH oversaw the project. WH, CZ, YD, DZ, YH, QT, OK, DM, JH contributed to sample collection and data suvey. LH and BC contributed to sample analysis. YX and YH wrote the first draft of the manuscript. All authors reviewed and commented on drafts of the manuscript.

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