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# Evaluation of Whole Blood Trace Element Levels in Chinese Children with Autism Spectrum Disorder

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## Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder, which has increased markedly during the last decades. Essential trace elements play an important role in neurological function and their imbalances are common in children with ASD. The objective of the present study was to investigate whole blood levels of trace elements including zinc (Zn), copper (Cu), iron (Fe), and magnesium (Mg) in Chinese children with ASD. In total, 113 children diagnosed with ASD and 141 age-matched and gender-matched neurotypical children, divided into two gender and age groups of preschool age (2–5 years old) and school (6–10 years old) age, were examined. The quantitative analyses of whole blood trace element contents were performed by using flame atomic absorption spectroscopy. In the present study, the children with ASD generally had lower whole blood levels of Zn than the neurotypical controls. No significant differences in the whole blood Cu, Zn/Cu ratio, Fe, or Mg was detected between the ASD group and the control group. It is notable that whole blood Fe level in boys with ASD was significantly higher than in girls with ASD, and was nearly significant when compared with the control level of boys. After stratification for age, a significant 6% decrease in whole blood Zn levels was detected in preschool-aged children with ASD as compared to the control values. However, this significant ASD-related change was not detected in school-aged children. The whole blood Zn level and Zn/Cu ratio were significantly increased in school-aged children than in preschool-aged children in both ASD and control group. In addition, school-aged children with ASD had a significantly higher level of whole blood Fe than preschool-aged children with ASD. The results of the present study suggest an association between whole blood levels of Zn in Chinese children with ASD.

**Keywords** Autism spectrum disorder · Trace elements · Zinc · Copper · Iron · Magnesium

## Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by early onset difficulties in social communication, along with unusually restricted, repetitive patterns of behaviors, interests, or activities. ASD has been increasing markedly during the last decades, affecting approximately 1 in 45 children in the USA, and it occurs three to four times more often in boys than in girls [1, 2].

Despite extensive research on ASD, the neurobiological and pathological mechanisms of ASD remain unclear [3]. The nutritional status has been reported to be altered in

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children with ASD [4]. A disturbance in the metabolisms of essential trace elements including zinc (Zn), copper (Cu), iron (Fe), and magnesium (Mg) is common in children with ASD [5–7]. The levels of trace elements are potentially modifiable and may influence the recommendations in ASD treatment [8–10]. Although plenty of studies have been conducted to determine the profile of essential trace elements in children with ASD, the current clinical data are still insufficient and inconsistent.

Zn is involved in enzyme function, nucleic acid metabolism, cell signaling, and apoptosis [11]. A growing number of studies have demonstrated a high prevalence of Zn deficiency in children with ASD [12–15]. However, the others failed to detect a significant ASD-related change in Zn levels [16, 17]. Recently, Babaknejad et al. conducted a meta-analysis of 12 published articles to explore the relationship between Zn metabolism and ASD. They found a significant statistical difference exists in plasma Zn concentration between controls and autistic patients but not in the hair, nail, or teeth Zn levels [18].

Since Cu excess can harm cells, the systematic and cellular Cu homeostasis is tightly regulated [19]. Dysregulation of Cu homeostasis may occur in patients with ASD. Although some studies have reported elevated serum Cu levels in ASD cases [8, 13], the others did not observe any significant changes [14, 20]. Moreover, the imbalance between Zn and Cu is proposed to be involved in ASD pathogenesis. Zn/Cu ratio was reported to correlate with the severity of symptoms associated with autism [7, 13], and was proposed as a biomarker in children with ASD [21, 22].

Fe plays important roles in the formation of hemoglobin, genetic repair, and central nervous system development. Deficiency of Fe could lead to irreversible cognitive impairments [23, 24]. On the other hand, like Cu, Fe overload is also toxic as it could impair DNA methylation [25] and could result in oxidative stress [26], which has been highlighted in the pathogenesis of ASD [27]. The existing data revealed that the level of Fe in different substrates from ASD patients may be decreased [28–30], increased [16], or similar to healthy developing children [31–33].

Mg deficiency has been linked to personality changes, including apathy, depression, and anxiety [34]. Previous studies have demonstrated contradictory findings of the changes in Mg content of children with ASD [28, 35–37].

Altogether, due to different substrates, inconsistent methods of measurement, contradictory data and relatively small sample sizes, it is still hard to explain the association between ASD and trace elements. Blood is the gold standard of clinical analysis of trace element status and is less variable. There is a lack of an exhaustive survey in the literature about the trace element levels in Chinese children with ASD. Only one study was previously performed to investigate the serum Zn and Cu levels in Chinese children with ASD [13]. Therefore, the aim of the present study was to evaluate whole blood Zn, Cu, Fe, and Mg concentrations in children with ASD from the east region of China.

## Methods

### Participants and Diagnostic Criteria

This cluster-matched case-control study was designed to assess if the status of the trace elements in the whole blood were changed in Chinese Han children with ASD aged 2 to 10 years. The study protocol was approved by the Ethics Committee of Zhejiang University. Written informed consent was obtained from the parents and/or from law tutors of each child.

Between January 1, 2015 and December 30, 2017, all participants were seen at the outpatient department of the Children's Hospital, Zhejiang University School of Medicine. They all received a series of physical examination, interviews, and observations conducted by a developmental pediatrician and a psychologist [38]. The diagnostic criteria of ASD were in agreements with the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). And typically developing children with age- and gender-matched coming for health examination were recruited as the controls. They had no developmental delays or social communication disorders. The exclusion criteria were as follows: (1) diagnosis of any chronic affective disease during the past 3 months; (2) frequent gastrointestinal problems (cyclic vomiting or chronic diarrhea) during the past 3 months; (3) vegetarian diet or food allergy; (4) use of any nutritional supplements or medication longer than 2 weeks during the past 3 months; (5) body mass index < 3rd centile or > 97th centile. The examined children all belong to the Chinese Han population and living in Zhejiang Province.

A total of 113 children diagnosed with ASD and 141 age- and gender-matched typically developing children were examined. The mean age of the controls and children with ASD was $4.94 \pm 2.11$ and $4.97 \pm 2.26$ years old, respectively. For further analysis of comparison, we divide all children into two genders (194 boys and 60 girls) and age groups: preschool age (2–5 years, $n = 184$) and school age (6–10 years, $n = 70$).

### Sample Collection and Trace Element Measurements

Whole blood samples were obtained from all examined children by using 4-ml lithium heparin vacuum blood collection tubes (20 international unit lithium heparin/ml). Before measurements, 40 μl whole blood of each sample was added into 1 ml sample diluent which was individually packed (Beijing Persee General Instrument Company Limited, Beijing, China), and then they were mixed by vibration. The quantitative analyses of trace element contents were performed by using flame atomic absorption spectroscopy on a multichannel atomic absorption spectrophotometer-MB5 (Beijing Persee General Instrument Company Limited, Beijing, China). We draw standard curves to calculate the concentrations of trace elements for every measurement. The detection

limits of the instrument for Zn, Cu, Fe, and Mg are 0.01 μmol/L, 0.01 μmol/L, 0.01 mmol/L, and 0.01 mmol/L, respectively.

## Data Analyses

Data were entered into Excel software and analyzed in the SPSS (version 23.0) software package. The values were expressed as mean ± standard deviation (SD). An independent *t* test was used to compare the differences of trace elements levels between two groups after the normal distributions of the values were tested. For correlation analyses between two variables in the ASD group, Spearman's correlation test was used. The *P* value of less than 0.05 was regarded as statistically significant.

## Results

The baseline characteristics of the whole cohort are summarized in Table 1. In the preschool-aged group, there were 104 control children (80 boys and 24 girls) and 80 children with ASD (62 boys and 18 girls), and the mean age of the control children and children with ASD was 3.87 ± 1.06 and 3.73 ± 1.02 years old, respectively. In the school-aged group, there were 37 control children (27 boys and 10 girls) and 33 children with ASD (25 boys and 8 girls), and the respective values were 7.96 ± 1.23 and 7.98 ± 1.44 years old.

The whole blood levels of Zn, Cu, Zn/Cu ratio, Fe, and Mg in children with ASD and the neurotypical controls are shown in Table 2. A significant ~6% decrease in the whole blood levels of Zn was detected in children with ASD as compared to the control values. However, no significant difference in whole blood Cu, Zn/Cu ratio, Fe, or Mg was detected between the two studied groups.

Table 3 showed the results of a further comparison analysis of different gender groups. When compared with the controls, the whole blood levels of Zn were significantly decreased by 5% and 7% in boys and girls with ASD, respectively. There was no significant difference in Cu, Zn/Cu ratio, Fe, or Mg between children with ASD and the controls in the same gender group. However, it is notable that the whole blood Fe level in boys with ASD was elevated and was nearly significantly ($P = 0.066$) higher when compared to the control level of boys. In the ASD group, the whole blood Fe level in boys with ASD was significantly increased by 5% than in girls. In the control group, there was no significant difference in whole blood trace element levels between boys and girls.

Table 4 showed the results of a further comparison analysis of different age groups. Among preschool-aged children, a significant 6% decrease in the whole blood Zn levels was detected in the ASD group as compared to the control values. Whereas among school-aged children, in spite of a 5–6% decrease in whole blood Zn content in the ASD group, the difference was not significant. In both two age groups, no significant difference in the whole blood concentrations of Cu, Zn/Cu ratio, Fe, or Mg was found between the control subjects and children with ASD. The whole blood Zn level was markedly increased by 8% and 9% in school-aged children when compared with preschool-aged children in the control and ASD groups, respectively. And the whole blood Zn/Cu ratio was significantly increased by 15% and 24% in school-aged children than in preschool-aged children in the control and ASD groups, respectively. In addition, the whole blood Fe content in school-aged children with ASD was significantly increased by 5% than in preschool-aged children with ASD.

**Table 2** Whole blood levels of trace elements (mean ± SD) in children with ASD and the controls

| Parameter | Control | ASD | *P* value |
|---|---|---|---|
| Age (years) | 4.94 ± 2.11 | 4.97 ± 2.26 | 0.906 |
| Zn (μmol/L) | 84.96 ± 12.09 | 80.18 ± 13.42 | 0.003[a] |
| Cu (μmol/L) | 20.87 ± 4.40 | 19.90 ± 5.09 | 0.103 |
| Zn/Cu ratio | 8.68 ± 2.74 | 8.10 ± 2.71 | 0.095 |
| Fe (mmol/L) | 8.34 ± 1.00 | 8.53 ± 0.97 | 0.119 |
| Mg (mmol/L) | 1.59 ± 0.18 | 1.59 ± 0.16 | 0.884 |

*ASD* autism spectrum disorder

[a] *P* value < 0.05 when compared with the control level

**Table 1** Demographic characteristics of children with ASD and the controls

| | Group of 2–5 years | | | Group of 6–10 years | | |
|---|---|---|---|---|---|---|
| | Control | ASD | In total | Control | ASD | In total |
| Case number | 104 | 80 | 184 | 37 | 33 | 70 |
| Age (mean ± SD years) | 3.87 ± 1.06 | 3.73 ± 1.02 | 3.81 ± 1.04 | 7.96 ± 1.23 | 7.98 ± 1.44 | 7.97 ± 1.33 |
| Gender | | | | | | |
| Boy (ratio) | 80 (77%) | 62 (78%) | 142 (77%) | 27 (73%) | 25 (76%) | 52 (74%) |
| Girl (ratio) | 24 (23%) | 18 (22%) | 42 (23%) | 10 (27%) | 8 (24%) | 18 (26%) |

*ASD* autism spectrum disorder

**Table 3** Whole blood trace element levels (mean ± SD) in boys and girls

| Parameter | Boys | | Girls | |
|---|---|---|---|---|
| | Control ($n = 107$) | ASD ($n = 87$) | Control ($n = 34$) | ASD ($n = 26$) |
| Age (years) | 4.85 ± 2.02 | 4.89 ± 2.15 | 5.23 ± 2.39 | 5.25 ± 2.60 |
| Zn (μmol/L) | 84.92 ± 12.54 | 80.59 ± 14.37 [a] | 85.08 ± 10.73 | 78.81 ± 9.72[a] |
| Cu (μmol/L) | 20.69 ± 4.14 | 19.71 ± 4.50 | 21.44 ± 5.17 | 20.50 ± 6.77 |
| Zn/Cu ratio | 4.25 ± 0.95 | 4.26 ± 1.14 | 4.19 ± 1.20 | 4.23 ± 1.53 |
| Fe (mmol/L) | 8.37 ± 1.01 | 8.64 ± 0.98 | 8.25 ± 0.89 | 8.19 ± 0.87[b] |
| Mg (mmol/L) | 1.60 ± 0.17 | 1.60 ± 0.16 | 1.57 ± 0.19 | 1.56 ± 0.16 |

*ASD* autism spectrum disorder

[a] $P$ value < 0.05 when compared with the control levels in the same gender group

[b] $P$ value < 0.05 when compared with the level of boy with ASD

Furthermore, the relationships between whole blood levels of Zn, Cu, Fe, and Mg and between these levels and age were assessed in children with ASD. Correlation analyses showed a modest positive correlation of levels between Fe and Mg ($r = 0.349$) as well as a weak positive correlation of levels between Zn and Cu ($r = 0.202$). In children with ASD, modest positive correlations between whole blood Zn level and age ($r = 0.343$) and between Zn/ratio and age ($r = 0.431$) were observed. Whole blood Cu levels ($r = -0.225$) and Fe levels ($r = 0.263$) negatively and positively correlated with age, respectively. There was no significant correlation between Mg levels and age in the ASD group.

## Discussion

The present study evaluated whole blood Zn, Cu, Fe, and Mg concentrations in Chinese children with ASD. The primary finding is that we observed a significant decrease in the whole blood Zn concentrations of children with ASD, in agreement with one previous whole blood study [14]. In further analysis of Zn levels in different gender groups, we found this significant ASD-related change existed in both boys and girls. Previous data obtained from other substrates of children with ASD revealed that Zn content of ASD cases may be decreased in serum sample [13], or similar to the controls in hair sample [16, 17]. Zn is primarily an intracellular nutrient, and erythrocyte Zn reflects a more long-term Zn status than serum Zn [39]. Furthermore, it has been demonstrated a strong correlation of Zn levels between whole blood and erythrocyte [12]. Therefore, change in whole blood Zn levels may be a reliable indicator of Zn-deficient state.

The underlying biochemical mechanisms of Zn deficiency in ASD children have not been fully elucidated. It may partly be explained by their unbalanced nutritional intake. Food selectivity, often referred to as "picky eating", is a common problem in children with ASD [4, 30, 40, 41]. Decreased dietary diversity in ASD has been associated with inadequate intake of protein, calcium, and vitamins [42]. Xia et al. previously conducted a three-day dietary survey in 111 Chinese children with ASD, and found that Zn intake in ASD cases did not meet the Dietary Reference Intakes requirements [43]. The inherited metabolic disturbances and low absorption ability due to pathological changes in the intestinal mucosa can also result in reduced Zn in ASD [5, 6, 44]. Moreover, Zn has an antagonistic relationship with heavy metals including lead and mercury [45], and one recent meta-analysis showed that children with ASD had elevated blood and erythrocyte levels of lead and mercury [46], therefore, the overload of these heavy metals may interfere with the absorption of Zn, though this effect is not well established. In addition, Zn is the most efficient producer of metallothionein, which is essential to heavy metal detoxification, and stress on the metallothionein system triggered by heavy metal exposure may cause faster use of Zn reserves [21]. Fluegge et al. speculated that the altered Zn levels in ASD may be a compensatory mechanism to counter the effects of air pollutant exposures [47].

After stratification for age, the whole blood Zn level increased markedly in school-aged children when compared with preschool-aged children in both control and ASD group, and the correlation analysis revealed a modest positive correlation between Zn level and age. Furthermore, we found that the significant ASD-related decrease of whole blood Zn level exists in preschool-aged children, but not in school-aged children. The occurrence of Zn deficiencies in ASD has been reported to be particularly pronounced in very young age [48]. And one recent longitudinal study has reported that food refusal could be improved during the follow-up of children with ASD [40]. Therefore, the intergroup difference in blood Zn content between ASD and the controls may be reduced in older children. In addition, the sample size of the school-aged group in the present study is relatively small, and it may limit our statistical power. A larger sample size study of school-aged children is warranted.

**Table 4** Whole blood trace element levels (mean ± SD) in children of different age group

| Parameter | Preschool age (2–5 years old) | | School age (6–10 years old) | |
|---|---|---|---|---|
| | Control ($n = 104$) | ASD ($n = 80$) | Control ($n = 37$) | ASD ($n = 33$) |
| Age (years) | 3.87 ± 1.06 | 3.73 ± 1.02 | 7.96 ± 1.23 | 7.98 ± 1.44 |
| Zn (μmol/L) | 83.12 ± 11.15 | 78.20 ± 13.00[a] | 90.15 ± 13.25[c] | 84.99 ± 13.41[b] |
| Cu (μmol/L) | 21.17 ± 4.46 | 20.46 ± 4.82 | 20.04 ± 4.18 | 18.54 ± 5.53 |
| Zn/Cu ratio | 4.07 ± 0.88 | 3.97 ± 0.92 | 4.69 ± 1.20[c] | 4.93 ± 1.59[b] |
| Fe (mmol/L) | 8.28 ± 0.89 | 8.40 ± 0.94 | 8.52 ± 1.20 | 8.86 ± 0.98[b] |
| Mg (mmol/L) | 1.60 ± 0.18 | 1.59 ± 0.16 | 1.56 ± 0.17 | 1.59 ± 0.16 |

*ASD* autism spectrum disorder

[a] $P$ value < 0.05 when compared with the control level in the same age group

[b] $P$ value < 0.05 when compared with the level of preschool-aged children with ASD

[c] $P$ value < 0.05 when compared with the control level of the preschool-aged children

There is a balance between the metabolism of Zn and Cu, and the disrupted Zn/Cu rhythmicity may occur in ASD [49]. Several previous studies have reported low Zn/Cu ratios or high Cu/Zn ratios resulting from decreased Zn and/or elevated Cu in ASD cases [7, 13, 21]. However, in the current study, we did not detect any significant change in whole blood levels of Cu or Zn/Cu ratio of children with ASD versus neurotypical controls. Intriguingly, we observed that the whole blood Cu level in the present study was slightly but not significantly decreased in children with ASD, in the same change direction of Zn. This finding is contradictory to the previous report of an antagonistic interaction of Zn and Cu [13] and may need more investigations.

In particular, the whole blood Fe level in boys with ASD had the highest level of whole blood Fe, which was significantly higher than that of girls with ASD, and was nearly significant when compared with the control level of boys. One previous study has revealed a significant ASD-related elevation of hair Fe content in boys but not in girls, indicating that the Fe level tended to be increased in boys with ASD [16]. However, the mechanism of this gender difference in peripheral Fe change of children of ASD is not clear and may need more research. It was previously hypothesized that excessive Fe might be associated with higher risk of ASD, based on the role of oxidative stress and intense immune reactions induced by free Fe radicals in the pathogenesis of ASD [50]. Moreover, Hfe mutation, which is associated with increased Fe uptake, has been recently implicated in DNA impairment, GABAergic dysfunction, and occurrence of repetitive behaviors in mice [25, 51].

There are some published studies that have reported significantly lower erythrocyte and serum Mg values in children with ASD [28, 35]. To our knowledge, we have investigated the blood Mg concentration of Chinese children with ASD for the first time, but we did not detect any significant difference in the whole blood Mg levels between the studied groups in the present study. Additionally, there was no significant correlation between whole blood Mg level and age in children with ASD, indicating that the Mg content is stable during growth.

## Strength and Limitations

This study is one of the first to look at trace elements and ASD in China. However, there were some limitations to the present study. Firstly, this is a cross-sectional survey and the collected data do not allow us to speculate on the duration of Zn deficiency in children with ASD. Secondly, we did not survey the dietary intake or meal-time behavior of examined children. Finally, we did not evaluate the correlation between the concentrations of trace elements and the symptom severity of children with ASD. Never the less, we have assessed the correlations between trace element levels and gender and age in the present study. And we are planning to incorporate the diagnostic tool of Autism Diagnostic Observation Schedule (ADOS) in our future research.

## Conclusions

Taken together, the results of the present study demonstrate that children with ASD are characterized by lower concentrations of serum Zn. Zn deficiency may be a complex mechanism and may be the target to the development of novel therapies for certain aspects of ASD. However, human studies using Zn or magnesium-pyridoxine supplementation in ASD patients so far have reported mixed findings [8, 9, 52]. Further investigations are warranted to investigate the effects of trace elements supplementation in ASD children and to provide mechanisms linking ASD and mineral homeostasis.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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