Association between Cu/Zn/Iron/Ca/Mg levels and Chinese children with cerebral palsy

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Research

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Abstract

Objective: It is well documented that the macro/trace elements are associated with neurodevelopment. We aimed to investigate the relationship between copper (Cu)/ zinc (Zn)/iron/calcium (Ca)/magnesium (Mg) levels and cerebral palsy (CP) risk in Chinese children by performing a meta-analysis.

Method: We searched the PubMed, Embase, Cochrane and Chinese WanFang databases from January 1985 to June 2019 to yield studies that met our predefined criteria. Fourteen studies were enrolled. Standard mean differences (SMDs) of

Cu/Zn/Iron/Ca/Mg levels between CP cases and controls were calculated using the fixed-effects model or the random-effects model, in the presence of heterogeneity.

95% confidence intervals (CI) were also computed.

Results: A total of 14 studies including 1452 CP cases and 1538 controls were involved. CP cases showed significantly lower levels of Cu, Zn, iron and Ca than those in controls (95% CI:-4.960- -2.302, $p<10^{-4}$; 95% CI:-4.061- -2.044, $p<10^{-4}$; 95% CI:-1.873 - -0.776, $p<10^{-4}$; 95% CI:-2.239- -0.487, $p=0.002$). Sensitivity analyses did not change the overall results markedly. No significant difference of Mg level between CP cases and controls (95% CI:-0.741-0.070, $p=0.105$) was observed. No marked publication bias was observed.

Conclusions: CP cases demonstrated significantly lower levels of Cu/Zn/iron/Ca than those in healthy controls. Lower levels of Cu/Zn/iron/Ca may be associated CP risk.

Frequent monitoring and early intervention may be needed.

Introduction

Cerebral palsy (CP), a group of motor disorders and cognitive disturbances, is a health concern in the newborns, particularly in the premature or low-birthweight neonates [1]. CP is likely to lead to the musculoskeletal problems, even epilepsy [2].

CP progression results in an increased morbidity and mortality, which indicates that early intervention for the risk factors of CP onset is of great importance. Strenuous efforts have been made to identify the risk factors for CP susceptibility. Birth asphyxia and genetic factors were involved in the development of CP [3]. In spite of the progress of the prenatal diagnosis and interventions, the prevalence of CP did not decline obviously, which indicated that CP may be a multi-factor disease, an in-depth investigation of other risk factors for CP is needed.

Trace elements status is closely associated with the immune system function via their effects on many biological processes, the well worked immune function required the micronutrients participating in cell metabolism and replication. For instance, leukocytes proliferation induced by acute infection was

impaired by insufficient supply of trace elements, including iron, zinc, magnesium and manganese [4]. Trace elements exert effects on the cellular transfer and the levels of other important nutrients [5]. For example, iron was an important constituent of hemoglobin, which carried the oxygen. It is also proved that certain trace elements affect the chemical synaptic transmission in the brain and peripheral central nervous system [6]. Cu and Zn play an important role in the activation of enzymes that are involved in catecholamine transmission. On the other hand, macro-elements, such as Ca and Mg play an important role in the physical development, including the brain. Ca and Mg exert effects in the transmission of neural stimuli.

Based on the fact that CP is essentially a neurological disorder, we speculated that macro/trace elements levels may reflect the susceptibility to CP in children. Some available evidence showed that certain trace elements, such as copper (Cu) deficiency was correlated with learning and behavior disorders [7]. Meanwhile, markedly lower level of zinc (Zn) was observed in severe CP compared with that in controls [8]. An in-depth understanding of the alterations of macro/trace elements levels in CP is of important significance provided that macro/trace elements levels may be an indicator for CP susceptibility.

Meta-analysis is a good way to pool the available evidence from single study to produce a comparatively robust result, which increases the statistical power significantly. Therefore, we conducted a meta-analysis with the aim of clarifying the differences of Cu/Zn/iron/Ca/Mg levels between CP and controls in Chinese children.

Materials And Methods

Search strategy

We performed the literature search in terms of the preferred reporting items for systematic reviews and meta-analysis guidelines [9], we searched the papers that reported the levels of Cu/Zn/iron/Ca/Mg, both in CP and controls from January 1985 to June 2019 by using PubMed, Embase, Cochrane and Chinese WanFang databases. We used the searched terms as follows: 1) macro/trace element, micronutrient, magnesium, Mg, calcium, Ca, iron, zinc, Zn, copper and Cu; 2) urine, serum and plasma; and 3) cerebral palsy, CP. We also reviewed the references of extracted literature. The paper with the larger number of participants was enrolled if the same subjects were recruited in more than one study.

Study selection criteria

1. Study design: case-control study
2. Case: cerebral palsy, control: healthy participants
3. Outcome of interest: Cu/Zn/iron/Ca/Mg levels in cases and controls

Data extraction

We collected the data of mean and standard deviation (SD) in Cu/Zn/Iron/Ca/Mg levels. We also extracted the study characteristics for enrolled investigations. Data were recorded as the followings: first author's last name; year of publication; ethnicity; number of cases and controls; confounding factors and testing method of Cu/Zn/Iron/Ca/Mg levels.

Statistical analyses

We used the standard mean difference (SMD) to test the differences of Cu/Zn/ Iron/Ca/Mg levels between CP cases and controls across studies. Heterogeneity of SMDs across studies was tested by using the Q statistic (significance level at $p < 0.05$). The I^2 statistic, which is a quantitative measure of inconsistency across studies, was also calculated. The SMDs were calculated using either fixed-effects models or, in the presence of heterogeneity, random-effects models. Sensitivity analysis was performed by omitting each study in turn. Potential publication bias was assessed by Begg's test at the $P < 0.05$ level of significance while the number of recruited studies were more than 5. All analyses were performed using STATA version 12.0 (Stata Corp, College Station, TX).

Results

Literature search

We firstly extracted 231 papers from the PubMed, Embase and Cochrane and Chinese Wan Fang databases. Most of these papers were removed due to the fact that they were not associated with CP or Cu/Zn/Iron/Ca/Mg levels. After full-text review of the remaining studies, three studies were excluded due to that the subjects were not Chinese. One study was excluded because it did not include control group. Finally, fourteen studies [10-23] were included in this meta-analysis. A flow diagram showing the study selection is presented in Figure 1.

Study characteristics

The characteristics of the fourteen enrolled studies are shown in Table 1. They were published between 1989 and 2016. Ten studies were about Cu, twelve for Zn, eight for iron, six for Ca, and four for Mg. Thirteen studies adjusted for confounding factors. All the participants were Chinese.

Cu level in CP and controls

A total of 875 CP cases and 811 controls were included in testing the Cu level. Atomic absorption spectroscopy (AAS) was used in testing the Ca level in five studies with anodic stripping (AS) in two studies, and inductively coupled plasma atomic emission spectrometry (ICP-AES) in two studies. CP cases showed markedly lower Cu level than that in controls (SMD= -3.631, 95% CI=-4.960- -2.302, $p < 10^{-4}$, Table 2, Figure 2). Sensitivity analysis did not change the overall results significantly (95% CI= -6.849- -0.754) . Publication bias was observed ($p = 0.001$). Exclusion of three studies (Hao et al 2000, Li et al 2003, Tang et al 2016) yielded little publication bias ($p = 0.051$).

Zn level in CP and controls

A total of 1352 CP cases and 1462 controls were included in testing the Zn level. AAS was used in testing the Zn level in seven studies with AS in two studies, and ICP-AES in two studies. CP cases showed markedly lower Zn level than that in controls (SMD= -3.052, 95% CI=-4.061- -2.044, $p<10^{-4}$, Figure 3). Sensitivity analysis did not change the overall results significantly (95% CI= -4.612- -1.023) . Publication bias was observed ($p=0.016$). Exclusion of two studies (Hao et al 2000, Li et al 2016) yielded little publication bias ($p=0.283$).

Iron level in CP and controls

A total of 773 CP cases and 749 controls were included in testing the iron level. AAS was used in testing the iron level in three studies with AS in two studies, ICP-AES in one study, turbidimetric method (TM) in one study. CP cases showed markedly lower iron level than that in controls (SMD= -1.325, 95% CI=-1.873- -0.776, $p<10^{-4}$, Figure 4). Sensitivity analysis did not change the overall results significantly (95% CI= -2.178- -0.519) . Publication bias was not observed ($p=0.063$).

Ca level in CP and controls

A total of 612 CP cases and 573 controls were included in testing the Ca level. AAS was used in testing the Ca level in three studies with AS in two studies. CP cases demonstrated significantly lower Ca level than that in controls (SMD= -1.363, 95% CI=-2.239- -0.487, $p=0.002$, Figure 5). Sensitivity analysis did not change the overall results significantly (95% CI= -2.617- -0.181) . Publication bias was not observed ($p=0.282$).

Mg level in CP and controls

A total of 512 CP cases and 497 controls were included in testing the Mg level. AAS was used in testing the Mg level in two studies with AS in another two. No marked difference of Mg level between CP cases and controls was noted (SMD=-0.741- 0.070, $p=0.105$).

Discussion

CP has attracted lots of attention from doctors and parents of patients due to its harms to neurological and motor systems in China. Identification of the potential risk factors for CP susceptibility is helpful for the prevention and treatment of CP. Our meta-analysis showed that CP cases demonstrated significantly lower levels of Cu, Zn, iron and Ca than those in controls, which indicated that the deficiency of Cu/Zn/iron/ Ca may be involved in the risk of CP. Early monitoring and intervention may be helpful for CP prevention and therapy.

Several facts may account for our findings. CP is a neurological disorder usually induced by preterm birth and infection. Metal ions are closely associated with the normal functioning of human body [24]. Trace elements deficiency is likely to cause the immune dysfunction, resulting in the increased risk of infection.

Cu is a key cofactor for various enzymes, such as Cu/Zn superoxide dismutase, which plays an important role for neurological development [25]. Cu is also involved in the redox reactions [26]. Cu deficiency affects the role of other cellular constituents involved in antioxidant activities, such as iron, selenium, and also play an important role in diseases in which oxidative stress is elevated. Oxidative stress was involved in the brain injury. Hence, the disorder of Cu may cause severe brain dysfunction. For example, higher level of Cu was associated with decreased risk of Parkinson's disease, which is the second most common neurodegenerative disease [27].

Zn is necessary for the survival of various cell types. Lots of enzymes exert the effects by creating bonds with Zn ions [28]. Zn plays a role in cell proliferation as an element of transcription factors and enzymes of DNA replication, Zn deficiency leads to a decline of Th1 immunity and promotes inflammatory reactions. Zn is also present throughout the central nervous system, playing a role in synaptic transmission, neuroregulation and neuroprotection [29]. Zn also promotes spinal cord injury recovery through upregulating Zn transporter-1 and brain-derived neurotrophic factors [30]. Zn inhibits free radical by promoting metallothionein production. Meanwhile, Zn is crucial for retinol-binding protein synthesis and vitamin A mobilization. Zn plays a role in removing the heavy metals from the body, such as Pb, As and Hg, which are implicated in the pathophysiology of Parkinson's disease [31]. Based on the comprehensive role of Zn, Zn disorder may result in the unpredictable injury including neurological lesions.

Iron is an important constituent of hemoglobin, which transfers oxygen. Iron deficiency leads to anemia. Thus, iron regulates and influences the activity of various organs, as well as the whole organism. Iron also exerts effects in the catalysis of enzymatic reactions [32]. Th cells maturation was impaired in children with iron-deficiency anemia and was regenerated by the supplementation of iron. Iron participates in the neurodevelopment [33]. Iron deficiency lowers the chances of recovery of the central nervous system and influences the children's adaptation ability.

Ca, an important constituent of bones, plays a vital role in the muscle contraction and relaxation, and regulates the electrical conduction system of the heart [34]. Ca also regulates the function of enzymes and is associated with the metabolism of other trace elements [35]. Mg, another important constituent of bones, is an antagonist of Ca, prevents excessive acetylcholine release and stimulation at the neuromuscular junction. Notably, we observed null difference of Mg between CP and controls, which may be due to that Mg was not directly related to the neurodevelopment. Further larger number of studies are needed to validate our findings.

Our findings supported the idea that nutritional status influences the neurodevelopment, neurocognitive performances, and later life health outcomes. Appropriate nutritional diet is important for lowering the adverse health consequences. Also, compared with included previously published single studies, our study was a pooled investigation with robust significances. Although the positive association between Cu/Zn/iron/Ca and CP provided novel insight for CP prevention and therapy, several limitations should be considered. First, the between-study heterogeneity may distort the final result, the random-effects model

decreased the influence of the heterogeneity. On the other hand, sensitivity analysis did not change the overall results, which indicated that our conclusion was comparatively robust. Second, the publication bias was noted for the association between Cu/Zn and CP, the exclusion of certain studies also removed the bias, strengthening our conclusions, indicating the outlier studies did not change the overall results. Finally, despite the significant differences of Cu/Zn/iron/Ca between CP and controls, the cause-effect relationship between trace elements levels and CP risk remains inconclusive. The enrolled participants were all children with a lower age, we speculated that trace elements deficiency may precede the CP onset. Due to the lack of specific age in our investigation, further larger number studies should be performed to address this issue.

In terms of our findings, the following issues should be addressed: 1) time-series analysis of the alteration of trace elements in CP, 2) longitudinal observation of the association between trace elements levels and CP progress, 3) clarification of the cause-effect relationship between trace elements status and CP risk in prospective studies.

In conclusion, our investigation indicates that CP cases demonstrated significantly lower levels of Cu/Zn/iron/Ca than those in healthy controls. Monitoring and intervention may be helpful for CP prevention and therapy.

Declarations

Contributions Wei Li conceptualized and designed this study, Haiquan Zhu collected the data, Song Mao analyzed the data and wrote this paper.

Fundings There is no funding to report in our analysis

Data availability The original extracted data can be obtained from the PubMed, Embase and Cochrane and Chinese Wan Fang databases (through searching the include article title for the full-text paper)

Conflict of interest There is no conflict of interest for all authors.

References

1. Spittle AJ, Morgan C, Olsen JE, Novak I, Cheong JLY. Early Diagnosis and Treatment of Cerebral Palsy in Children with a History of Preterm Birth. *Clin Perinatol*. 2018;45(3):409–20. (PMID: 30144846).
2. Hollung SJ, Bakken IJ, Vik T, Lydersen S, Wiik R, Aaberg KM, Andersen GL. Comorbidities in cerebral palsy: a patient registry study. *Dev Med Child Neurol*. 2019 Jul 4. doi: 10.1111/dmcn.14307 (PMID: 31273772).
3. Sun L, Xia L, Wang M, Zhu D, Wang Y, Bi D, Song J, Ma C, Gao C, Zhang X, Sun Y, Wang X, Zhu C, Xing Q. Variants of the OLIG2 Gene are Associated with Cerebral Palsy in Chinese Han Infants with Hypoxic-Ischemic Encephalopathy. *Neuromolecular Med*. 2019;21(1):75–84. (PMID: 30178266).

4. Elmadfa I, Meyer AL. **The Role of the Status of Selected Micronutrients in Shaping the Immune Function.** *Endocr Metab Immune Disord Drug Targets*. 2019 May 28. doi: 10.2174/1871530319666190529101816 (PMID: 31142256).
5. Chatelain M, Gasparini J, Haussy C, Frantz A. Trace Metals Affect Early Maternal Transfer of Immune Components in the Feral Pigeon. *Physiol Biochem Zool*. 2016;89(3):206 – 12. (PMID: 27153130).
6. Wendołowicz A, Stefańska E, Ostrowska L. Influence of selected dietary components on the functioning of the human nervous system. *Rocz Panstw Zakl Hig*. 2018;69(1):15–21. (PMID: 29517182).
7. Zheng J, Jiang R, Chen M, Maimaitiming Z, Wang J, Anderson GJ, Vulpe CD, Dunaief JL, Chen H. Multi-Copper Ferroxidase-Deficient Mice Have Increased Brain Iron Concentrations and Learning and Memory Deficits. *J Nutr*. 2018;148(4):643–9. (PMID: 29659961).
8. Schoendorfer NC, Vitetta L, Sharp N, DiGeronimo M, Wilson G, Coombes JS, Boyd R, Davies PS. Micronutrient, antioxidant, and oxidative stress status in children with severe cerebral palsy. *JPEN J Parenter Enteral Nutr*. 2013;37(1):97–101. (PMID: 22610980).
9. Moher D, Liberati A, Tezlaiff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg*. 2010;8(5):336–41. (PMID: 20171303).
10. Li Z. Study on serum trace elements levels in 168 cerebral palsy cases. *Chinese Journal of Misdiagnostics*. 2003;3(11):1674–5. (in Chinese).
11. Yuan H, Zhang G, Long Y, Chen D, Tao Y. Study on elements (Ca, Zn, Fe, Cu, Mg) of blood in 128 CP. *GuangDong Weiliang Yuansu Kexue*. 2007;14(5):13–5. (in Chinese).
12. Liang H, Du W, Zhang X, Xing Q, Wang J, Jiang Y. Study on urine zinc and copper in children with cerebral palsy. *Trace elements health research*. 1999;16(3):26–7. (in Chinese).
13. Chen X, Gao Z, Sun L, Dong X, Zhang T. **To research the microelement and immune function in children with cerebral palsy. Selected Thesis of international TCM psychology and systems bioinformatics.** 2010;3:379–381 (in Chinese).
14. Wang H, Chen X, Li S, Wei C, Li Y, Li J. Study on the change of serum zinc and copper levels in children with cerebral palsy. *Jia Mushi medical college research*. 1989;12(3):270–1. (in Chinese).
15. Hu Y. Study on the serum trace elements in children with cerebral palsy. *Medical Journal of Chinese People's Health*. 2008;20(21):2513–5. (in Chinese).
16. Li Q, Du C, Ren X. Analysis of serum zinc level in children with cerebral palsy. *Chinese Journal of Practical Nervous Disease*. 2016;19(12):95–6. (in Chinese).
17. Hao Q, Gao Y, Li F. Study on the change of serum iron in children with cerebral palsy. *China clinical rehabilitation*. 2004;8(21):4276–6. (in Chinese).
18. Li M, Wu J, Zhang H, Yan J, Chen Q, Xiong C. **Nutritional status and intervention for children with cerebral palsy.** 2014;20(12):1150–1152 (in Chinese).
19. Li L. Association between trace elements and growth/development in children with cerebral palsy. *Chinese Journal of Practical Nervous Disease*. 2016;19(11):79–80. (in Chinese).

20. Peng G. Study on the calcium and bone density in children with cerebral palsy. *Studies of Trace elements health*. 2005;22(6):16–7. **(in Chinese)**.
21. Yang H, Li Q, Wang K, Wang J, Tang H, Wang L, Wang X. **Association between zinc and children with cerebral palsy**. 2009;31(2):166–167 **(in Chinese)**.
22. Hao Q, Guo X, Wu T, Dong J. **Association between serum trace elements and cause of Children with cerebral palsy**. 2000;8(4):109–111 **(in Chinese)**.
23. Tang Y. Study on the change of serum trace elements and oxidative stress in children with severe cerebral palsy. *Chinese Journal of Practical Nervous Diseases*. 2016;19(18):93–4. **(in Chinese)**.
24. Polyudova TV, Eroshenko DV, Korobov VP. **Plasma, serum, albumin, and divalent metal ions inhibit the adhesion and the biofilm formation of *Cutibacterium (Propionibacterium) acnes***. *AIMS Microbiol*. 2018;4(1):165–72. **(PMID: 31294208)**.
25. Lasienne J, Komine O, Fujimori-Tonou N, Powers B, Endo F, Watanabe S, Shijie J, Ravits J, Horner P, Misawa H, Yamanaka K. **Neuregulin 1 confers neuroprotection in SOD1-linked amyotrophic lateral sclerosis mice via restoration of C-boutons of spinal motor neurons**. *Acta Neuropathol Commun*. 2016 Feb 18;4:15 **(PMID: 26891847)**.
26. Ognik K, Cholewińska E, Juśkiewicz J, Zduńczyk Z, Tutaj K, Szlęzak R. The effect of copper nanoparticles and copper (II) salt on redox reactions and epigenetic changes in a rat model. *J Anim Physiol Anim Nutr (Berl)*. 2019;103(2):675–86. **(PMID: 30618103)**.
27. Kim MJ, Oh SB, Kim J, Kim K, Ryu HS, Kim MS, Ayton S, Bush AI, Lee JY, Chung SJ. Association of metals with the risk and clinical characteristics of Parkinson's disease. *Parkinsonism Relat Disord*. 2018;55:117–21. **(PMID: 29891430)**.
28. Khan MF, Kundu D, Hazra C, Patra S. A strategic approach of enzyme engineering by attribute ranking and enzyme immobilization on zinc oxide nanoparticles to attain thermostability in mesophilic *Bacillus subtilis* lipase for detergent formulation. *Int J Biol Macromol*. 2019;136:66–82. **(PMID: 31181278)**.
29. Justice JA, Manjooran DT, Yeh CY, Hartnett-Scott KA, Schulien AJ, Kosobucki GJ, Mammen S, Palladino MJ, Aizenman E. Molecular Neuroprotection Induced by Zinc-Dependent Expression of Hepatitis C-Derived Protein NS5A Targeting Kv2.1 Potassium Channels. *J Pharmacol Exp Ther*. 2018;367(2):348–55. **(PMID: 30190339)**.
30. Li X, Chen S, Mao L, Li D, Xu C, Tian H, Mei X. **Zinc Improves Functional Recovery by Regulating the Secretion of Granulocyte Colony Stimulating Factor From Microglia/ Macrophages After Spinal Cord Injury**. *Front Mol Neurosci*. 2019 Feb 1;12:18 **(PMID: 30774583)**.
31. Tamegart L, Abbaoui A, Makbal R, Zroudi M, Bouizgarne B, Bouyatas MM, Gamrani H. Crocus sativus restores dopaminergic and noradrenergic damages induced by lead in Meriones shawi: A possible link with Parkinson's disease. *Acta Histochem*. 2019;121(2):171–81. **(PMID: 30573341)**.
32. Stoyanovsky DA, Tyurina YY, Shrivastava I, Bahar I, Tyurin VA, Protchenko O, Jadhav S, Bolevich SB, Kozlov AV, Vladimirov YA, Shvedova AA, Philpott CC, Bayir H, Kagan VE. Iron catalysis of lipid

peroxidation in ferroptosis: Regulated enzymatic or random free radical reaction? Free Radic Biol Med. 2019;133:153–61. :**(PMID: 30217775)**..

33. Wang Y, Wu Y, Li T, Wang X, Zhu C. Iron Metabolism and Brain Development in Premature Infants. Front Physiol. 2019;10:463. **(PMID: 31105583)**.
34. Nishikawa K, Dutta S, DuVall M, Nelson B, Gage MJ, Monroy JA. Calcium-dependent titin- thin filament interactions in muscle: observations and theory. J Muscle Res Cell Motil. 2019 Jul 9. doi: 10.1007/s10974-019-09540-y **(PMID: 31289970)**.
35. Gaglianone RB, Bloise FF, Ortiga-Carvalho TM, Quirico-Santos T, Costa ML, Mermelstein C. **Comparative study of calcium and calcium-related enzymes with differentiation markers in different ages and muscle types in mdx mice.** Histol Histopathol. 2019 Jul 5;**18145**. doi: 10.14670/HH-18-145 **(PMID: 31274171)**.

Tables

Characteristics of studies enrolled in this meta-analysis

et al 1989	CC	Chinese	42/30	102.51±25.46/121.28±29.34 ug%	Age,Gender	AAS
et al 1999	CC	Chinese	20/20	0.0578±0.0162/0.0762±0.016 ug/mL	Age,Gender	AAS
al 2000	CC	Chinese	60/32	1.16±0.25/23.14±1.26 umol/L	Age,Gender	ICP-AES
2003	CC	Chinese	168/152	0.50±0.27/0.71±0.23 ug/mL	Age,Gender	ICP-AES
t al 2007	CC	Chinese	128/128	14±3.71/15±3.26 mmol/L	Age,Gender	AS
l 2008	CC	Chinese	131/130	3.60±0.54/3.80±0.51 ug/mL	Age,Gender, Habits	AAS
t al 2010	CC	Chinese	36/20	14.71±3.79/22.06±1.93 umol/L	Age,Gender	AAS
2014	CC	Chinese	53/60	1.071±0.231/1.139±0.372 ug/mL	Age,Gender	AAS
2016	CC	Chinese	217/219	18.38±3.90/19.23±4.07 umol/L	Age,Gender	AS
t al 2016	CC	Chinese	20/20	8.76±1.48/20.31±3.79 umol/L	Age,Gender	-
et al 1989	CC	Chinese	42/30	81.64±12.47/96.19±9.61 ug%	Age,Gender	AAS
et al 1999	CC	Chinese	20/20	0.558±0.22/0.775±0.195 mg/L	Age,Gender	AAS
al 2000	CC	Chinese	60/32	3.81±0.96/24.43±1.07 umol/L	Age,Gender	ICP-AES
2003	CC	Chinese	168/152	3.04±1.05/5.83±1.76 ug/mL	Age,Gender	ICP-AES
t al 2007	CC	Chinese	128/128	78±24/81±29 mmol/L	Age,Gender	AS
l 2008	CC	Chinese	131/130	2.80±1.20/3.50±1.40 ug/mL	Age,Gender, Habits	AAS
t al 2009	CC	Chinese	277/311	3.5±0.9/4.7±1.3 umol/L	Age,Gender	AAS
t al 2010	CC	Chinese	36/20	67.96±15.06/119.14±29.70 umol/L	Age,Gender	AAS
2014	CC	Chinese	53/60	0.889±0.295/1.229±0.318 ug/mL	Age,Gender	AAS
2016	CC	Chinese	200/340	47.19±1.53/67.77±1.60 umol/L	Age,Gender	AAS
2016	CC	Chinese	217/219	58.42±14.47/61.6±12.11 umol/L	Age,Gender	AS
t al 2016	CC	Chinese	20/20	9.57±2.24/12.65±2.56 umol/L	Age,Gender	-
					-	
2003	CC	Chinese	168/152	428±43/451±61 ug/mL	Age,Gender	ICP-

						AES
al 2004	CC	Chinese	20/20	6.37±2.03/15.57±2.12 umol/L	Age,Gender	TM
t al 2007	CC	Chinese	128/128	41±7.29/53±6.37 mmol/L	Age,Gender	AS
l 2008	CC	Chinese	131/130	141.01±54.94/161.48±51.45 ug/mL	Age,Gender, Habits	AAS
t al 2010	CC	Chinese	36/20	7.09±0.87/8.15±1.14 umol/L	Age,Gender	AAS
2014	CC	Chinese	53/60	0.757±0.218/1.141±0.307 ug/mL	Age,Gender	AAS
2016	CC	Chinese	217/219	7.31±1.29/7.55±0.89 mmol/L	Age,Gender	AS
t al 2016	CC	Chinese	20/20	10.28±2.56/20.75±6.21 umol/L	Age,Gender	-
t al 2005	CC	Chinese	80/56	253.15±68.84/288.44±87.28 mmol/L	Age,Gender	AAS
t al 2007	CC	Chinese	128/128	1.01±0.22/1.29±0.31 mmol/L	Age,Gender	AS
l 2008	CC	Chinese	131/130	18.21±4.80/65.60±22.11 ug/mL	Age,Gender, Habits	AAS
t al 2010	CC	Chinese	36/20	1.31±0.127/1.83±0.26 umol/L	Age,Gender	AAS
2016	CC	Chinese	217/219	1.81±0.17/1.87±0.23 mmol/L	Age,Gender	AS
t al 2016	CC	Chinese	20/20	1.48±0.41/1.82±0.51 mmol/L	Age,Gender	-
t al 2007	CC	Chinese	128/128	1.01±0.31/1.12±0.40 mmol/L	Age,Gender	AS
l 2008	CC	Chinese	131/130	49.35±8.73/56.22±6.43 ug/mL	Age,Gender, Habits	AAS
t al 2010	CC	Chinese	36/20	1.47±0.518/1.45±0.08 umol/L	Age,Gender	AAS
2016	CC	Chinese	217/219	1.49±0.23/1.51±0.19 mmol/L	Age,Gender	AS

ontrol, Cu=copper, Zn=zinc, Ca=calcium, Mg=magnesium, ICP-AES=inductively coupled plasma atomic emission ry, AAS=atomic absorption spectroscopy, AS=Anodic stripping, TM=turbidimetric method.

le 2 Association between the status of trace elements and cerebral palsy

Trace elements	Studies	Q test	Model selected	SMD(95% CI)	P-value
	P-value				
Cu					
Overall	10	$<10^{-4}$	Random	-3.631(-4.960- -2.302)	$<10^{-4}$
Zn					
Overall	12	$<10^{-4}$	Random	-3.052(-4.061- -2.044)	$<10^{-4}$
Iron					
Overall	8	$<10^{-4}$	Random	-1.325(-1.873- -0.776)	$<10^{-4}$
Ca					
Overall	6	$<10^{-4}$	Random	-1.363(-2.239- -0.487)	0.002
Mg					
Overall	4	$<10^{-4}$	Random	-0.336(-0.741-0.070)	0.105

per, Zn=zinc, Ca=calcium, Mg=magnesium, SMD=standard mean difference.

Figures

Figure 1

Flow diagram of study selection

Figure 2

Difference of Cu level between CP and control

Figure 3

Difference of Zn level between CP and control

Figure 4

Difference of iron level between CP and control

Figure 5

Difference of Ca level between CP and control