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Comparison of serum essential trace metals between patients with schizophrenia and healthy controls

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ABSTRACT

Preclinical and clinical studies have suggested that essential trace metals (ETMs) play an important role in the pathophysiology of brain-based disorders, including schizophrenia. This case-control study aimed to evaluate the association between ETMs and schizophrenia, and to further examine the association between ETMs and clinical characteristics in schizophrenia. One-hundred and five (n = 105) subjects who meet DSM-IV criteria for schizophrenia between the ages of 18 and 40 were recruited for the study. One hundred and six (n = 106) age- and sex-matched healthy controls (HCs) were recruited for comparison. Serum concentrations of seven ETMs [i.e. iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), manganese (Mn), nickel (Ni) and molybdenum (Mo)] were evaluated using inductively coupled plasma mass spectrometry, which allows for the quantitative analysis of multiple ETMs at a single time point. Compared to HCs, serum concentrations of Mn and Mo were significantly lower in patients with schizophrenia. In contrast, serum concentrations of Fe and Ni were significantly higher in patients with schizophrenia. Additionally, correlations between specific ETMs and metabolic parameters (particularly those related to liver and renal function) were found in patients with schizophrenia, and the correlations between every two ETMs in HCs were widely interrupted. Differential levels of selected ETMs (i.e., Mn, Mo, and Ni) were identified between patients with schizophrenia and HCs following adjustment for potential confounders. The findings here should therefore be evaluated in future studies.

1. Introduction

Schizophrenia is a severe, complex, and disabling mental disorder that affects approximately 1% of the global population [1]. Schizophrenia is characterized by a set of positive, negative, and cognitive symptoms, and is associated with significant morbidity and mortality [2,3]. Despite significant advancements in the understanding of the epidemiology, neurobiology, and genetics of schizophrenia, the causal mechanisms underlying the development of schizophrenia remain unknown [4]. Evidence from preclinical and clinical studies have implicated a role for trace elements in the pathophysiology of disparate brain-based disorders, namely schizophrenia. [5–7].

The human body requires essential trace metals (ETMs) during development and adult life to sustain disparate metabolic and physiological processes. ETMs, for example, iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), manganese (Mn), nickel (Ni) and molybdenum (Mo) are required for proper immune system regulation, neurodevelopment, body growth, and cognitive function [8]. ETMs, namely Zn and Mn

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have also been reported to influence emotional processing by modulating aspects of neural transmission [9,10]. Replicated evidence has supported the association between schizophrenia and changes in serum ETM levels. Hitherto, the functional role of ETMs in schizophrenia are not elucidated in literature [11,12]. Some studies have reported reduced Zn concentrations in hair and plasma in subjects with schizophrenia compared to healthy controls (HCs) [13,14] whereas other studies have reported comparable Zn concentrations between individuals with and without schizophrenia [7,15]. Zn has also been associated with cognitive dysfunction (i.e., learning and memory impairments) in preclinical models [16]. Other ETMs have also been implicated in the pathophysiology of schizophrenia. For example, prepartum maternal Fe deficiency has been demonstrated to increase the risk of developing schizophrenia in offspring [17,18]. Finally, excess Mn levels in the brain- has a neurotoxic potential - has been reported to confer risk for developing schizophrenia [19]. Associations between levels of other ETMs (i.e., Co, Ni, Mo) and schizophrenia have also been reported [11,20,21].

Taken together, the mechanistic and clinical relevance of selected ETMs (i.e. Fe, Zn, Cu, Co, Mn, Ni, Mo) in schizophrenia remain obscure. Herein, we conducted a case-control study to compare serum ETM levels between adults with and without schizophrenia, and to investigate the associations between ETMs and different clinical characteristics in schizophrenia. The findings of the present study provide a more comprehensive understanding of the pathophysiology of ETMs in schizophrenia, and suggest that adjunctive ETM modulation could serve as new approaches to therapeutic interventions in patients with the disease.

2. Methods

2.1. Study population

We recruited 105 patients meeting Diagnostic and Statistical Manual, Fourth Edition (DSM-IV)-defined criteria for schizophrenia that had not taken any antipsychotic drugs for a minimum of 1 month prior to hospitalization, and 106 age- and sex-matched HCs without any current and/or past psychiatric diagnosis. All subjects were recruited concurrently from the same district at the Weifang Mental Health Center in Shandong Province, China between November 2015 and September 2016. Subjects were excluded based on the following criteria: (1) < 18 or > 40 years old; (2) history of occupational exposure in the heavy metals industry; (3) diagnosis of diabetes, hyperlipidemia, cardiovascular disease, or any other severe medical condition; (4) presence of comorbid psychiatric conditions (e.g., substance use disorders); and (5) current or recent (i.e., within post-partum period of 12 months) pregnancy (suspected or confirmed) and/or breastfeeding.

The study protocol was reviewed and approved by the Ethics Review Committee of the Health Science Center, Peking University (IRB00001052-12065). All participants provided written and informed consent.

2.2. Serum sample preparation and metal analysis

Blood samples (approximately 5 mL) were collected from subjects between 7 A.M. and 9 A.M. following a 12-hour, overnight fast. After coagulation at room temperature for ~30 min, serum samples were isolated by centrifugation at 3000 *g* for 15 min at 4 ℃ and immediately stored at -80 ℃ until use. A direct dilution method was used for the metal preparation, i.e. 100 μL of each serum sample were transferred to a quartz tube, added 0.1 mL indium (2 ng/mL) as an internal standard element, then added 1.8 mL 1% nitric acid and mixed [22]. The concentrations of seven ETMs (i.e. Fe, Zn, Cu, Co, Mn, Ni and Mo) were analyzed in randomized order using inductively coupled plasma mass spectrometry (ICP-MS) (ELAN DRCII; PerkinElmer, USA). The main parameters of ICP-MS were as follows: nebulizer gas flow, 0.97 L/min; auxiliary gas flow, 1.86 L/min; plasma gas flow, 17.0 L/min; power of radio frequency generator, 1.15 KW; dwell time, 100 ms; mode peak, hopping; resolution, 0.7-0.9 au m. To avoid spectral interferences, Dynamic Reaction Cell (DRC) mode was used for the determination of Fe and Mn.

Mixed standard chemical substances for all seven ETMs with 7-point calibration curves were used for quantification. The preparation of standard chemical substances was the same as serum samples. Metal element measurements were based on the most abundant isotope for each element to avoid interference, and Level II (ClinChek® Serum Controls, REF 8884) of the certified reference material (CRM) was used for quality assurance. As shown in Supplemental Table 1, all measured metal concentrations were included in the range of their CRM with the recovery from 92.56% to 103.15%. The mixed standard chemical substances were also used to monitor the stability and repeatability of the actual samples (i.e. these were analyzed once for every 10 study samples).

2.3. Statistical analysis

All statistical analyses were conducted using SPSS ver. 22.0 (SPSS Inc., Chicago, IL, USA). Body Mass Index (BMI) was calculated using the formula weight (kg) divided by the square of height in meters *m* (kg/m^2). Descriptive statistics were performed, with continuous variables summarized using the mean and standard deviation (SD) or median and interquartile range (IQR), while categorical variables were summarized using frequencies and proportions. Statistical significance between groups was tested using the Chi-Squared (χ^2) test or Fisher's exact test for categorical variables and t-test, or Mann-Whitney *U* test for continuous variables. The median value of the ETM concentrations in HCs was used as the cut-off value in the dose-response analysis. Unconditional logistic regression models were used to explore the association between schizophrenia and ETMs. The variables of age, sex and BMI were included as potential covariates in the unconditional logistic regression model. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using maximum likelihood methods. A partial correlation analysis on ranks (i.e., Spearman correlation) was used to calculate the correlation coefficients between ETM levels and metabolic parameters. The Benjamini-Hochberg false discovery rate (FDR) control was implemented to correct for multiple comparisons. A two-tailed P-value or FDR *q*-value of < 0.05 was used to indicate statistical significance.

3. Results

3.1. Demographic and clinical characteristics

Clinical and demographic characteristics for all subjects are shown in Table 1. Of the 105 recruited patients with schizophrenia, 24 (22.9%) were drug-naïve and experienced their first-episode of schizophrenia. The remaining 81 patients had recurrent and/or chronic schizophrenia and had not taken any antipsychotic drugs for a minimum of 1 month prior to hospitalization. The mean age was 29.3 years (SD = 5.6), 61 subjects were female (64.2%), and all subjects were of Chinese Han ethnicity. The mean BMI was 24.0 kg/m^2 (SD = 4.2) and 13 (13.3%) subjects were obese (i.e., BMI > 28). The mean age of onset for schizophrenia was 23.1 (SD = 5.6) years, and the median (IQR) duration of illness was 6.1 (2.8, 10.1) years. There was no statistical difference between cases and HCs in age (P = 0.920), sex (P = 0.367), BMI (P = 0.437), cigarette smoking status (P = 0.355), and alcohol consumption (P = 0.315).

Table 2 displays the blood biochemistry analysis results for metabolic parameters. No significant differences were found between cases and HCs on lipid metabolism and glucose metabolism parameters [i.e., fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC)] (all P > 0.05). In contrast, significant differences were found between

Table 1
Distribution of the demographic characteristics of cases and HCs.

Characteristics	SCZ (n = 105)	HC (n = 106)	*P-value*
Age (year), mean (SD)	29.3 (5.6)	30.65 (4.6)	0.920[a]
Sex(n, %)			
Male/ Female	44 (35.8)/ 61(64.2)	38 (41.9)/ 68 (58.1)	0.367[b]
BMI (kg/m^2), mean (SD)	24.0 (4.2)	23.6 (4.2)	0.437[a]
Obese (n, %)	13 (13.3)	12 (11.3)	0.196[b]
Smoker (n, %)	13 (12.4)	9 (8.5)	0.355[b]
Drinker (n,%)	11 (15.1)	16 (10.5)	0.315[b]
PANSS, median (IQR)			
Total score	81.0 (72.0, 91.0)	____	
Positive symptoms	21.0 (18.0, 26.0)	____	
Negative symptoms	18.0 (13.0, 22.5)	____	
General psychopathology	41.0 (37.0, 48.0)	____	
First-episode *vs* Relapse (n, %)	24/81 (22.9/ 77.1)	____	
Age of onset (years), mean (SD)	23.1 (5.6)	____	
Duration of illness (years), median (IQR)	6.1 (2.8, 10.1)	____	

[a] Calculated by two-tailed independent-samples t-test.
[b] Pearson's chi-square test.

the groups on all liver function parameters. In particular, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were higher in cases compared to HCs, whereas albumin (ALB) and total protein (TP) levels were lower. Of the renal function parameters, both creatinine (CREA) and uric acid (UA) levels were higher in cases.

3.2. Serum ETM levels

Serum ETM concentrations for all participants are listed in Table 3. Between-group analyses showed that the concentrations of Mn (cases: 1.61 ng/mL, IQR: 1.34–1.94 ng/mL; HCs:2.70 ng/mL, IQR: 2.35–2.94 ng/mL, $q < 0.001$) and Mo (cases: 1.30 ng/mL, IQR: 1.10–1.57 ng/mL; HCs:1.49 ng/mL, IQR: 1.19–1.86 ng/mL, $q = 0.009$) were significantly lower in cases compared to HCs, whereas concentrations of Fe (cases: 1.31 μg/mL, IQR: 1.09–1.51 ng/mL; HCs:1.16 ng/mL, IQR: 0.96–1.41 ng/mL, $q = 0.012$) and Ni (cases: 3.66 ng/mL, IQR: 3.05–4.16 ng/mL; HCs:3.19 ng/mL, IQR: 2.61–4.00 ng/mL, $q = 0.009$) were significantly higher in cases. There were no significant differences between groups in the concentrations of the remaining ETMs (i.e. Zn, Cu and Co) (all $q > 0.05$). Comparing first-episode schizophrenia cases and recurrent schizophrenia cases to HCs, we observed lower concentrations of Mn and lower concentrations of Mn and Mo in first-episode schizophrenia cases and recurrent schizophrenia cases, respectively. Moreover, the analysis revealed elevated Fe, and Ni concentrations among recurrent schizophrenia cases compared to HCs. There were no significant differences of the detected seven ETMs between first-episode and recurrent cases of schizophrenia (all $q > 0.05$).

We calculated the ORs of the seven ETMs associated with schizophrenia using dichotomized ETMs (Table 4). Median values of the ETM concentrations in the HCs were used as the cut-off values. The ORs of Zn, Mn and Mo were < 1.0, while all the other ORs were > 1.0. Univariate analyses revealed that elevated serum concentrations of Fe and Ni and reduced serum concentrations of Mn and Mo were significantly associated with schizophrenia. The significant differences of Zn, Mo, Ni concentrations were maintained after adjusting for age, sex, BMI, smoking and alcohol consumption. The dose-response relationships between ETMs and schizophrenia were depicted in Supplemental Fig. 1. The serum ETM concentrations were classified into four levels by quartiles of HCs. The adjusted ORs revealed that serum levels of Mn, Zn and Mo were negatively associated with schizophrenia, and that elevated serum levels of Ni and Co were associated with schizophrenia.

3.3. Correlations between serum ETMs levels within cases and HCs

Correlations of ETM levels within cases and HCs were conducted using Spearman correlation analyses (Fig. 1, the detailed results of the analysis are shown in Supplemental Table 2). Within the HC group, most of the ETMs were positively correlated with each other. A negative correlation was only found between Fe and Co ($r = -0.210$, $P = 0.031$). In the case group, these correlations were reduced.

3.4. Correlations between ETM levels and metabolic parameters in cases

We calculated correlations between ETM levels and metabolic parameters in patients with schizophrenia (Table 5). The analyses revealed significant positive correlations between serum concentrations of Cu and FBG ($r = 0.345$) or TG ($r = 0.312$) or TP ($r = 0.213$), Zn and ALB ($r = 0.216$), Mn and ALB ($r = 0.250$) or TP ($r = 0.290$), and Fe and AST ($r = 0.242$) or ALT ($r = 0.261$), all $P < 0.05$. The serum concentration of Co was negatively correlated with CREA ($r = -0.294$) and UA ($r = -0.242$). In addition, a significant negative correlation was observed between Ni and ALB ($r = -0.258$) or TP ($r = -0.269$) in the case group, all $P < 0.05$. In addition, the situations of correlations between ETM levels and metabolic parameters in HCs were quite different from those of the cases (Supplemental Table 3).

4. Discussion

Essential trace metals are important components of enzymes and hormones that are potentially involved in metabolic processes of the central nervous system (CNS) [23]. Using inductively coupled plasma

Table 2
Comparison of metabolic parameters between cases and HCs in blood biochemistry analysis.

Classification	Variables	SCZ	HC	*q-values*[*]
Lipid metabolism	TG (mmol/L); median (IQR)	1.0 (0.7, 1.5)	0.9 (0.6, 1.4)	0.379[a]
	TC (mmol/L); median (IQR)	4.5 (3.9, 5.1)	4.3 (3.8, 4.9)	0.163[a]
Glucose metabolism	FBG (mmol/L); median (IQR)	5.1 (4.7, 5.6)	5.3 (5.0, 5.6)	0.096[a]
Liver function	AST (U/L); median (IQR)	19.0 (15.0, 25.0)	16.0 (13.0, 20.0)	0.003[a]
	ALT (U/L); median (IQR)	18.0 (15.0, 22.8)	16.0 (11.0, 22.0)	0.005[a]
	ALB (g/L); mean (SD)	38.4 (3.2)	44.8 (2.8)	< 0.001[b]
	TP (g/L); mean (SD)	70.3 (7.4)	74.4 (6.4)	< 0.001[b]
Renal function	UREA (mmol/L) ; mean (SD)	4.4 (1.3)	4.5 (0.9)	0.361[b]
	CREA (umol/L); median (IQR)	76.0 (65.5, 89.0)	69.0 (59.0, 83.0)	0.003[a]
	UA (umol/L); median (IQR)	309.0 (250.0, 418.5)	266.0 (216.5, 327.5)	< 0.001[a]

[a] Calculated by two-tailed Mann-Whitney U tests.
[b] Calculated by two-tailed independent-samples t-test.
[*] q-values were false discovery rate (FDR) corrections for P-values.

Table 3
Comparisons of ETM levels in serum samples from cases and HCs.

ETMs	All Cases (n = 105)		First-episode (n = 24)		Recurrent (n = 81)			HC (n = 106)
	median (IQR)	*q-values* [a*]	median (IQR)	*q-values* [b*]	median (IQR)	*q-values* [c*]	*q-values* [d*]	median (IQR)
Zn (μg/mL)	0.88 (0.76, 0.98)	0.163	0.83(0.74,0.99)	0.297	0.88(0.77,0.98)	0.233	> 1.000	0.90 (0.77, 1.10)
Mn(ng/mL)	1.61 (1.34, 1.94)	< 0.001	1.70(1.63,2.47)	< 0.001	1.52(1.31,1.88)	< 0.001	0.056	2.70 (2.35, 2.94)
Cu(μg/mL)	0.85 (0.77, 1.02)	0.137	0.81(0.71,0.88)	0.852	0.86(0.77,1.04)	0.052	0.413	0.83 (0.71,0. 95)
Fe (μg/mL)	1.31(1.09, 1.51)	0.012	1.29 (1.07,1.50)	0.231	1.31(1.13,1.51)	0.021	> 1.000	1.16 (0.96, 1.41)
Co (ng/mL)	0.40 (0.35, 0.48)	0.251	0.41(0.34,0.47)	0.875	0.40(0.36,0.49)	0.250	> 1.000	0.38 (0.32, 0.50)
Ni(ng/mL)	3.66 (3.05, 4.16)	0.009	3.63(3.13,4.08)	0.196	3.66(2.99,4.2)	0.019	0.976	3.19 (2.61, 4.00)
Mo (ng/mL)	1.30 (1.10, 1.57)	0.009	1.34(1.05,1.66)	0.289	1.30(1.10,1.57)	0.019	> 1.000	1.49 (1.19, 1.86)

IQR, inter-quartile range, unit: ng/mL serum.
[a]comparsion between all cases and HCs, calculated by two-tailed Mann-Whitney *U* tests.
[b]comparsion between cases with first-episode and HCs, calculated by two-tailed Mann-Whitney *U* tests.
[c]comparsion between cases with recurrent and HCs, calculated by two-tailed Mann-Whitney *U* tests.
[d]comparsion between first-episode cases and recurrent cases, calculated by two-tailed Mann-Whitney *U* tests.
*q-values were FDR corrections for *P*-values.

Table 4
ORs of ETMs in serum between cases and HCs.

ETMs	Univariate OR(95%CI) [a]	*q-value**	Adjusted OR(95%CI) [b]	*q-value**
Zn	0.721 (0.419, 1.242)	0.278	0.611 (0.340, 1.099)	0.140
Mn	0.061 (0.024, 0.150)	< 0.001	0.058 (0.023, 0.147)	< 0.001
Cu	1.234 (0.718, 2.120)	0.466	1.158 (0.653, 2.054)	0.615
Fe	2.088 (1.194, 3.651)	0.021	2.002 (1.074, 3.732)	0.051
Co	1.396 (0.805, 2.387)	0.333	1.460 (0.801, 2.663)	0.253
Ni	2.182 (1.245, 3.823)	0.021	2.174 (1.205, 3.922)	0.023
Mo	0.522 (0.300, 0.908)	0.037	0.453 (0.250, 0.821)	0.032

[a] Calculated by an unconditional Logistic regression model.
[b] Adjusted OR and 95%CI were calculated by an unconditional Logistic regression model adjusting for age, sex, BMI, smoking and alcohol consumption.
* *q*-values were FDR corrections for *P*-values.

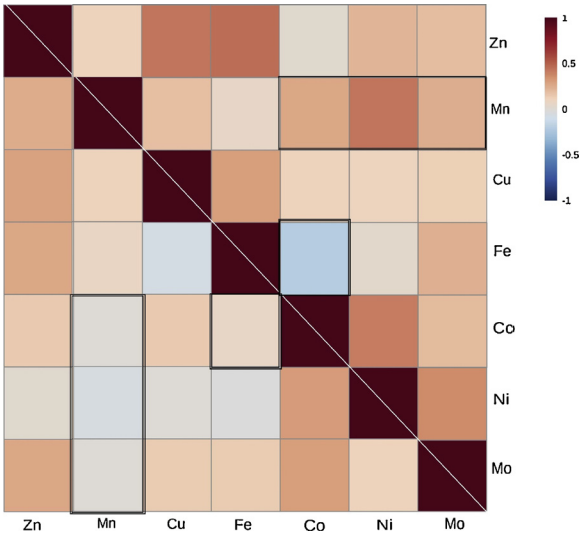

Fig. 1. Spearman correlations of ETM levels in cases and HCs. The lower-left corner represents the correlations of ETMs in cases while the upper-right corner represents the correlations of ETMs in HCs.

mass spectrometry, our case-control study compared the serum concentrations of seven ETMs between unmedicated patients with schizophrenia and HCs. The findings suggest that higher concentrations of Fe and Ni, and lower concentrations of Mn and Mo may be associated with schizophrenia. Some of the correlations between the aforementioned specific ETMs in HC group were absent in the case group. In addition, significant correlations between specific ETMs and metabolic parameters (particularly those related to liver and renal function) were found in patients with schizophrenia. These findings support the hypothesis that changes in serum levels of certain ETMs are associated with schizophrenia.

Iron plays an essential role in routine cellular functioning, as well as in DNA and neurotransmitter synthesis, which is demonstrated by its ability to act as a coenzyme in the oxygenation of neurotransmitters and enzymes [24]. Research has suggested that iron may also play an important role in brain development and cognition [25,26]. A recent study demonstrated that high levels of iron may lead to enhanced oxidative stress and subsequent neuronal death [27]. To support this, a mechanism has been proposed wherein H_2O_2 is converted into OH (a neurotoxic oxygen radical) via Fenton reactions in the presence of high levels of iron [28]. Thus, the observation of elevated Fe concentrations in patients with schizophrenia may be indicative of neurotoxicity and implicate oxidative stress in the pathophysiology of schizophrenia. In addition, two liver function parameters (i.e. AST and ALT) were found to be positively correlated with the serum Fe concentrations in patients with schizophrenia. Moreover, both parameters were higher in schizophrenia cases compared to HCs. Excess iron has been shown to mediate liver toxicity and hepatic injury via mechanisms of oxidative stress [29,30]. It should be noted that higher serum Fe concentrations in schizophrenia has not been uniformly reported. For example, an earlier study reported lower levels of Fe in patients with schizophrenia and suggested that the iron deficiency may affect the dopaminergic system [15]. We assumed that Fe has a threshold for human health, the inconsistent findings of the results in previous literatures need further exploration [15,29–31]. In addition, there was no longer significant difference of higher iron levels in schizophrenia after adjusting age, sex, BMI, smoking and alcohol consumption both for dichotomized and polytomized data in current study. Thus, our current results should be considered with caution and the health effects of iron in different populations is worth further exploration.

Studies have identified diverse toxic properties of Ni in the body, including toxic effects on hematopoietic, immune, reproductive, and pulmonary systems. Nickel has also been shown to have neurotoxic, genotoxic, nephrotoxic, hepatotoxic and carcinogenic effects [32]. No previous studies have reported on the potential role of Ni in the pathogenesis of schizophrenia, thus our findings indicating that excess Ni may be associated with an increased risk of developing schizophrenia provides insight into the field. This is supported by evidence from clinical and preclinical studies, which demonstrate that excess Ni leads to the formation of free radicals in tissues. Free radical formation in tissues has been associated with the modification of DNA bases, enhanced lipid peroxidation, and increased oxidative stress in tissues [33]. Lower ALB and TP were also found patients with schizophrenia compared to HCs, and there was a negative correlation between Ni and ALB or TP in patients with schizophrenia. Together, our findings suggest a potential hepatotoxic role of excess Ni in schizophrenia.

As a vital component of manganese superoxide dismutase (MnSOD),

Table 5
Spearman correlations between ETM levels and metabolic parameters in patients with schizophrenia. (For interpretation of the references to colour in the table, the reader is referred to the web version of this article.)

Correlations	Zn	Mn	Cu	Fe	Co	Ni	Mo
FBG	0.065	0.070	0.345**	0.035	0.082	-0.074	0.067
TG	0.132	0.037	0.312**	0.003	-0.183	-0.009	0.013
TC	0.133	0.076	0.147	0.053	0.001	-0.109	0.053
AST	0.073	0.069	-0.007	0.242*	-0.017	0.011	0.068
ALT	0.033	0.048	-0.041	0.261**	-0.125	-0.007	0.076
ALB	0.216*	0.250*	-0.013	0.180	-0.133	-0.258**	-0.083
TP	0.162	0.290**	.213*	0.064	-0.041	-0.269**	-0.167
UREA	-0.129	0.112	0.027	0.075	-0.190	-0.149	-0.159
CREA	0.095	0.090	0.024	0.167	-0.294**	-0.109	-0.020
UA	0.183	0.093	0.183	-0.044	-0.242*	-0.123	-0.032

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
The red background represents the positive correlations between the two compared variables, while the blue background represents the negative correlations.

Mn is essential for normal brain and nerve function [34]. The results of a meta-analysis conducted in a Chinese population revealed that decreased MnSOD activity may be associated with increased risk of chronic schizophrenia [35]. In line with this, we identified lower Mn concentrations in patients with schizophrenia compared to HCs, thus hypothesizing that lower Mn concentrations are associated with reduced MnSOD activity. Other case-control studies measuring Mn concentrations in plasma [15] and scalp hair samples [13] have reported similar results. In addition, Mn is a cofactor for arginase and is required to support its functional activity. Consistent with this, preclinical studies have suggested that Mn-deficiency suppresses hepatic arginase activity [36]. Under normal conditions, there is competition between arginase and nitric oxide synthase. Consequently, low levels of Mn (acting as an arginase cofactor) have been associated with an increased production of nitric oxide (NO) which this study proved was observed in patients with schizophrenia [37,38]. These findings are consistent with our observation of lower arginine levels in patients with schizophrenia compared to HCs [39]. We also observed the positive correlations between Mn and two liver function parameters - ALP and TP- which were found in patients with schizophrenia. The current study provides clues for exploring the role of Mn in the abnormal metabolism of arginase and liver function in schizophrenia, which has already been studied in animal models [40].

The transition element Mo is biologically inactive as a single agent and it requires interaction with other elements and/or proteins to be active [41]. The concentration of serum Mo in schizophrenia has been scarcely reported in previous studies. However, recent animal studies conducted in sheep have indicated that Mo deficiency promotes the accumulation of select dietary purines into the CNS [42,43], leading to astrocyte dysfunction, changes in neuromodulation, and eventually, irreversible CNS disease (such as Parkinson's disease) [43]. Our findings suggest that Mo may confer a protective benefit against schizophrenia, as serum levels were significantly lower in case patients compared to HCs. Therefore, we hypothesise that the active form of Mo may have a neuroprotective effect, and the metabolic balance of Mo may be dysregulated in schizophrenia.

Similar to our observations of dose-response effects, a recent meta-analysis reported that significantly reduced serum Zn concentrations were found in individuals with schizophrenia compared to controls [44]. Zinc deficiency has been reported to be associated with cognitive development by alterations in attention, activity, neuropsychological behavior and motor development [45]. Therefore, further investigation about the role of Zn in schizophrenia is warranted, particularly due to the potential association between serum Zn levels and brain glutamatergic system function [16].

No associations were found between schizophrenia and serum concentrations of Cu and Co. Previous research indicated that Cu plays a role in the synthesis of dopamine and norepinephrine by participating in the tyrosine and dopamine β-hydroxylase metabolic processes [26,46]. A significant study conducted in a Chinese population reported that antipsychotic treatment effectively reduced the concentration of Cu in schizophrenic patients to levels comparable to that of controls [46]. In addition, repeated evidence also reported higher concentrations of Cu in patients with schizophrenia [7,46,47]. Therefore, further investigation of the association between serum Cu and schizophrenia is warranted, particularly due to the potential association between serum Cu levels and dopamine metabolism. Next steps with respect to research vistas are to identify the roles of the elements reviewed herein and understand mechanistically how they affect neural structures that subserve domain-based psychopathology such as cognitive impairment and reward disturbance.

5. Limitations

Several limitations should be addressed when interpreting the results of our study. Firstly, no conclusions can be made regarding causality between ETMs and schizophrenia due to our case-control design. Secondly, total metal concentrations were measured without specificity regarding the specific form and/or valence state of the metal. It is possible that varying forms and/or valence states of a metal may influence metabolism differently. In addition, due to high heterogeneity in the presentation and characterization of schizophrenia, the generalizability of our findings is limited. Finally, our results report differences in ETM concentrations between cases and HCs in peripheral blood, which may not necessarily reflect differences in ETM concentrations in the brain.

6. Conclusion

The results of our case-control study suggest that patients with schizophrenia have reduced serum concentrations of Mn and Mo, and increased serum concentrations of Ni compared to HCs. In addition, the correlations between various ETMs in HCs were largely reduced in the patients with schizophrenia. Our results provide the basis for hypothesizing that ETMs may be relevant to the pathophysiology of schizophrenia.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

Wang JY, Yan LL and Cao B conceived and designed the study; Cao B, Yan LL, Ma JH, and Jin M collected the data and performed the statistical analysis; Li N, Yan LL, Cao B, Pan ZH, Brietzke E, McIntyre RS, and Park C contributed to the discussion; Li N, Wang JY, McIntyre RS, Park C, Nozari Y, Brietzke E, Kazmierczak OP, Lui LMW and Pan ZH revised the paper. All authors have read and approved the final version of this article.

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Institutional review board statement

This study was reviewed and approved by the Eths Review Committee of Public Health at Peking University Health Science Center (IRB00001052-14071).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2018.10.009.

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