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Association between serum trace element concentrations and valproic acid-induced hepatotoxicity in pediatric patients with epilepsy

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Abstract: Valproic acid (VPA), a commonly used antiepileptic drug, may cause hepatotoxicity during its clinical use. However, the mechanisms underlying VPA-associated hepatotoxicity remain unclear. In this study, 137 age-matched epileptic patients receiving long-term VPA treatment were enrolled, and the blood samples were collected for liver function, trace element concentrations and oxidative stress tests. The results revealed that patients with VPA-induced hepatotoxicity had higher concentrations of iron (Fe, $P < 0.001$), and lower concentrations of cobalt (Co) and selenium (Se) than those in the control group ($P = 0.036$ and $P < 0.001$, respectively). In addition, multiple regression analysis indicated that the Fe concentration was positively associated with transferase activities and oxidative stress parameters (glutathione and thiobarbituric acid-reactive substances, $P < 0.05$), while the concentrations of Co and Se were negatively correlated with transferase activities and oxidative stress parameters ($P < 0.05$). Moreover, logistic regression analysis indicated that the Fe concentration was correlated with a greater risk for hepatotoxicity ($P = 0.001$, OR: 2.387), whereas the concentrations of Co ($P = 0.038$, OR: 0.889) and Se ($P = 0.001$, OR: 0.813) were negatively correlated with VPA-associated hepatotoxicity. These results clarified that certain trace elements (Fe, Co and Se) may contribute to the pathogenesis of VPA-associated hepatotoxicity via the oxidative stress pathway.

Keywords: epilepsy, valproic acid, trace elements, hepatotoxicity, pediatric patients

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INTRODUCTION

Valproic acid, also known as VPA, is the main antiepileptic drug used for the treatment of epilepsy and bipolar disorder (Mishra *et al.*, 2021). Despite being effective and generally tolerated, long-term VPA therapy has been linked to hepatotoxicity (Young *et al.*, 2022). However, the underlying mechanisms of VPA-induced hepatotoxicity remain partially unknown.

Trace elements, a series of rare but essential elements, execute vital functions in human metabolism and endocrinological processes (Calderón Guzmán *et al.*, 2019). However, trace elements produce double-edged effects on human health. Perturbations in trace element status (including alternations in concentrations, proportions, and distributions of trace elements) may result in liver diseases (Nangliya *et al.*, 2015; Himoto & Masaki, 2020). For example, selenium (Se), cobalt (Co) and copper (Cu) concentrations may be involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) in adults (Wang *et al.*; Tinkov *et al.*, 2024). Moreover, a previous study confirmed that alternations in zinc (Zn) and iron (Fe) promote the NAFLD progression from steatosis to steatohepatitis in methionine-choline-deficient (MCD) rats and patients (Ghamarchehreh *et al.*, 2016; Kim *et al.*, 2020; Palladini *et al.*, 2022). Importantly, long-term VPA treatment may change the status of trace elements (especially the serum zinc and selenium concentrations) (Jia *et al.*, 2020). Hence, the alternations in trace element status may contribute to VPA-associated hepatotoxicity, but the underlying mechanism remains unclear.

Oxidative stress is a special pathophysiological condition, induced by an imbalance between the generation of reactive oxygen species (ROS) and the endogenous antioxidant defense system (van der Pol *et al.*, 2019). Accumulating studies have demonstrated that changes in certain trace elements are related to the occurrence of oxidative stress (Noshin *et al.*, 2021; Yang *et al.*, 2021). In detail, Cu was confirmed to induce oxidative stress in chicken hepatocytes and patients with hepatocellular carcinoma. (Geetha *et al.*, 2009; Yang *et al.*, 2019). In addition, Zn deficiency is associated with cellular oxidative stress and involved in the development of myocardial infarction and ischemia/reperfusion injury (Choi *et al.*, 2018). Moreover, serum Fe and Se concentrations are also correlated with oxidative stress in patients with coronary artery disease and migraine (Noshin *et al.*, 2021; Talaie *et al.*, 2022). Furthermore, previous studies indicated that oxidative stress was linked with a series of diseases (e.g. neurodegenerative diseases, cancer and liver diseases) (Jelic *et al.*, 2021; Sadasivam *et al.*, 2022; Teleanu *et al.*, 2022). Crucially, increasing evidence which suggests that oxidative stress is being connected to the pathogenesis of

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VPA-associated liver diseases via deficiency in ROS elimination and overexpression of CD36 and DGAT2 (Ma *et al.*, 2019a; Ma *et al.*, 2020). Hence, we hypothesize that trace element-related oxidative may contribute to the development of VPA-associated hepatotoxicity. However, research data on the relationships among oxidative stress, trace elements and VPA-associated hepatotoxicity are limited. Whether the effects of trace elements on VPA-associated liver diseases are dependent on oxidative stress needs to be explored.

The current study was performed to systematically investigate the associations between trace elements and VPA-associated hepatotoxicity in pediatric patients with epilepsy. The results revealed that certain trace elements (Fe, Co and Se) concentrations were associated to serum transaminase activities and contributed to the pathogenesis of VPA-associated hepatotoxicity via the oxidative stress pathway. This study provides a novel understanding of the mechanism for VPA-induced hepatoxicity.

MATERIAL AND METHODS

Patient cohort

In this study, 137 age-matched patients (age range: 1-11 years) were recruited at Yantai Yuhuangding Hospital. All patients were diagnosed with epilepsy according to the practical clinical classification of epilepsy (Fisher *et al.*, 2014). In addition, all patients underwent VPA-based antiseizure treatment for more than three months and were divided into a hepatotoxicity cases group (aminotransferase activities exceeding two-fold the upper limit of normal with or without prolonged in prothrombin time, 45 patients) and a control group (with normal liver function, 92 patients) according to liver function tests (Chen *et al.*, 2019; Xu *et al.*, 2019). Moreover, patients with pre-existing hepatotoxicity and potential causes of hepatotoxicity (such as metabolic syndrome, hepatitis and HIV-positive) were excluded from this study.

Blood collection and laboratory assays

After fasting overnight (minimal fasting period of 6 h for children up to the age of 3 years), venous blood (5 mL) was collected from each patient. Biochemical indicators (such as the activities of AST, ALT, GGT, ALP and concentrations of TP and ALB) were determined with a biochemistry analyzer (Au5800, Beckman Coulter, USA) within 30 min. The concentrations of thiobarbituric acid-reactive substances (TBARS) and glutathione (GSH) were determined as described previously (Ma *et al.*, 2024).

Quantification of VPA and trace element concentrations

For each patient, just before the last VPA administration, 3-5 mL of venous blood was collected to determine the steady-state VPA concentration. The VPA concentration was quantified as described previously (Ma *et al.*, 2019b). Certain trace elements [including cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), strontium (Sr) and zinc (Zn)] were performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) at ICP-2060T (Skyray Instrument, Suzhou, China) according to the protocols given by the manufacturer.

STATISTICAL ANALYSIS

The statistical analyses for this study were conducted via SPSS software (version 20.0; IBM, USA). The demographic characteristics, liver function tests and trace element concentrations were performed using Student's t test. The statistical significance of concomitant drugs between the cases and control group was determined by Fisher's exact test. Logistic regression was used to evaluate the risk factors for valproic acid-associated hepatotoxicity. The data are expressed as the means ± standard deviations, and a P-value less than 0.05 indicated statistical significance.

RESULTS

Demographic characteristics of the pediatric patients with VPA-based therapy

A total of 137 age-matched pediatric patients with VPA-based therapy were recruited at Yuhuangding Hospital. These patients were divided into a control group (92 patients) and a cases group (45 patients) according to the liver function test. As shown in table 1, the basal activities of GGT, ALT, AST and the concentrations of TBiL, GSH and TBARS significantly differed between the cases and control groups ($P < 0.05$). Meanwhile, no significant differences were observed in demographic characteristics (such as age, height, body weight or BMI) and concentrations of TP and ALB between both groups ($P > 0.05$).

Comparison of dose regimens between hepatotoxicity and control group

In the present study, the dose regimens and concomitant drugs used for each patient were recorded and summarized in table 2. In detail, the dose, concentration and adjusted concentration of VPA did not differ between cases and control group ($P > 0.05$). Besides, carbamazepine (five patients), clonazepam (three patients), lamotrigine (seven patients), levetiracetam (four patients), topiramate (five patients) and oxcarbazepine (one patient) were combined with VPA administration. However, no significant differences were detected in concomitant drugs between the cases and control groups ($P > 0.05$). Moreover, we also analyzed the demographic characteristics and liver function results of patients receiving VPA monotherapy (excluding patients with concomitant treatment, Supplementary table S1). These results suggest that dose regimens and concomitant drugs treatment may not be directly associated with VPA-associated hepatotoxicity.

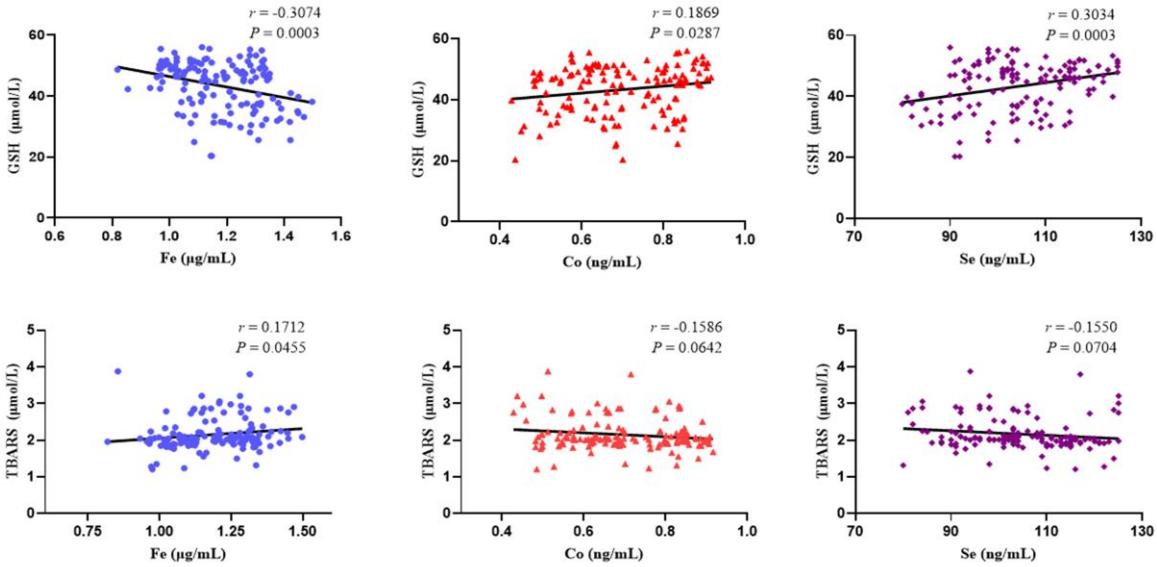

Fig.1: Association between trace elements concentrations (iron, cobalt and selenium) and oxidative stress levels (glutathione and thiobarbituric acid-reactive substances concentrations)

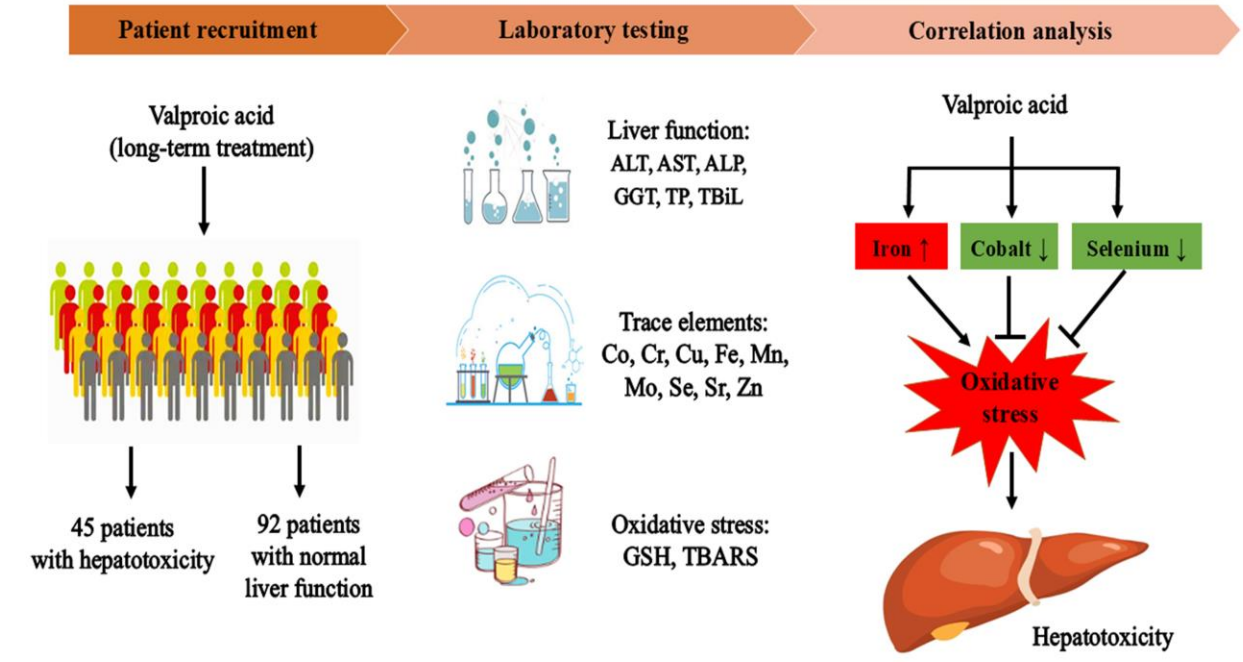

Fig.2: Schematic representation of the role of glutamate-glutamine cycle in valproic acid-associated hepatotoxicity. Red box: risk factors for VPA-induced hepatotoxicity. Green box: protective factors for VPA-induced hepatotoxicity

Table 1: Demographic data of patients in the hepatotoxicity and control groups

Demographic characteristics	Cases	Control	*P* value
Number of patients	45 (32.8 %)	92 (67.2 %)	N/A
Age (years)	4.73 ± 3.52	4.46 ± 2.89	0.630
Height (cm)	104.38 ± 38.18	101.10 ± 27.43	0.608
Body weight (kg)	22.87 ± 10.46	20.85 ± 9.85	0.272
BMI (kg/m^2)	20.55 ± 5.58	19.82 ± 7.19	0.551
TP (g/L)	67.45 ± 8.38	66.47 ± 5.36	0.353
ALB (g/L)	41.83 ± 9.77	42.50 ± 3.99	0.659
TBiL (μmol/L)	9.78 ± 6.30	6.61 ± 2.54	0.002
GGT (U/L)	45.85 ± 34.41	19.46 ± 16.14	＜0.001
ALP (U/L)	190.91 ± 124.28	187.40 ± 58.90	0.858
ALT (U/L)	83.98 ± 41.98	13.39 ± 7.27	＜0.001
AST (U/L)	97.60 ± 66.95	26.52 ± 9.01	＜0.001
GSH(μmol/L)	33.58 ± 4.92	48.04 ± 3.93	＜0.001
TBARS (μmol/L)	2.32 ± 0.44	2.10 ± 0.39	0.005

The bolded data indicated $P < 0.05$

Table 2: Comparison of valproic acid dosage regimens in hepatotoxicity and control group

Dose regimens	Cases (n = 45)	Control (n = 92)	*P* value
VPA concentration (μg/mL)	63.93 ± 28.38	57.18 ± 21.04	0.161 [a]
VPA daily doses (mg/kg)	25.77 ± 15.34	25.83 ± 21.11	0.986 [a]
Adjusted VPA concentration [(μg/mL)/(mg/kg)]	3.17 ± 2.38	3.39 ± 2.58	0.627 [a]
Carbamazepine use	2 (4.4%)	3 (3.3%)	0.537 [b]
Clonazepam use	1 (2.2%)	2 (2.2%)	0.701 [b]
Lamotrigine use	3 (6.7%)	4 (4.3%)	0.429 [b]
Levetiracetam use	1 (2.2%)	3 (3.3%)	0.605 [b]
Topiramate use	2 (4.4%)	3 (3.3%)	0.537 [b]
Oxcarbazepine use	0 (0%)	1 (1.1%)	0.674 [b]

[a] Statistical significance was determined by Student's t-test
[b] Statistical significance was determined by the Fisher's exact test

Table 3: Comparison of serum trace elements in hepatotoxicity and control groups

Trace elements	Cases (n = 45)	Control (n = 92)	*P* value
Co (ng/mL)	0.66 ± 0.13	0.71 ± 0.13	0.036
Cr (ng/mL)	1.62 ± 0.48	1.70 ± 0.53	0.423
Cu (μg/mL)	1.28 ± 0.20	1.24 ± 0.17	0.838
Fe (μg/mL)	1.26 ± 0.13	1.14 ± 0.13	＜0.001
Mn (ng/mL)	1.89 ± 0.36	1.94 ± 0.25	0.436
Mo (ng/mL)	1.57 ± 0.22	1.63 ± 0.18	0.117
Se (ng/mL)	97.58 ± 10.55	107.48 ± 10.15	＜0.001
Sr (ng/mL)	37.62 ± 10.23	40.35 ± 8.38	0.099
Zn (μg/mL)	0.84 ± 0.10	0.87 ± 0.12	0.061

The bolded data indicated $P < 0.05$

Table 4: Multiple regression analysis for the association between serum trace elements and liver function tests

Trace elements	GGT		TBiL		AST		ALT	
	β	*p*	β	*p*	β	*p*	β	*p*
Co	-0.134	0.119	-0.081	0.356	-0.210	0.012	-0.246	0.003
Cr	-0.003	0.969	0.007	0.941	0.029	0.724	0.016	0.846
Cu	-0.049	0.568	0.013	0.884	0.041	0.622	-0.029	0.716
Fe	0.297	0.001	0.189	0.053	0.266	0.002	0.294	0.001
Mn	0.044	0.607	0.033	0.703	-0.116	0.158	-0.119	0.139
Mo	0.033	0.696	0.137	0.113	-0.114	0.164	-0.069	0.387
Se	-0.057	0.518	-0.129	0.153	-0.181	0.035	-0.219	0.009
Sr	-0.076	0.381	-0.122	0.172	-0.036	0.668	-0.082	0.317
Zn	-0.058	0.503	0.009	0.914	0.081	0.562	0.392	0.079

The bolded data indicated $P < 0.05$

Table 5: Logistic regression analysis of risk factors for valproic acid-associated hepatotoxicity.

Variables	Regression Coefficient	P Value	Exp (B)	95% CI
Co	-0.930	0.038	0.889	0.735 – 1.071
Cr	-0.287	0.420	0.750	0.373 –1.509
Cu	0.208	0.837	1.231	0.711 – 1.843
Fe	2.381	0.001	2.387	1.530 – 3.617
Mn	-0.558	0.377	0.572	0.166 – 1.171
Mo	-0.494	0.118	0.224	0.035 – 1.459
Se	-1.092	0.001	0.813	0.677 – 0.950
Sr	-0.037	0.102	0.964	0.922 – 1.007
Zn	0.228	0.068	1.040	0.917 – 1.276

The bolded data indicated $P < 0.05$

Comparison of trace elements concentrations between hepatotoxicity and control group

To explore the potential mechanisms for VPA-associated hepatotoxicity, the concentrations of certain trace elements were analyzed. As presented in table 3, the concentration of Fe was significantly higher in hepatotoxic patients than that in control patients ($P < 0.001$). Besides, the concentrations of Co and Se was significantly lower in cases group than those in control group ($P = 0.036$ and $P < 0.001$, respectively). Moreover, no significant differences were found in the concentrations of Cr, Cu, Mn, Mo, Sr and Zn between the two groups ($P > 0.05$). Furthermore, we also analyzed the concentrations of these trace elements in patients with valproic acid monotherapy (table S2). These results suggest that the concentrations of Fe, Co and Se may be linked with VPA-associated hepatotoxicity.

Influence of trace elements on liver function and oxidative stress parameters in epileptic patients

In order to elucidate associations between the changes in trace elements (Fe, Co and Se) and altered hepatotoxicity markers (GGT, ALT, AST and TBiL), multiple regression analysis was performed (table 4). The regression models demonstrated that serum Fe concentration was positively associated with the activities of GGT, ALT and AST ($P = 0.001$, $P = 0.001$ and $P = 0.002$, respectively). Besides, the serum Co and Se concentrations were negatively correlated with AST ($P = 0.012$ and $P = 0.035$) and ALT activities ($P = 0.003$ and $P = 0.009$, respectively). Moreover, although the TBiL concentration in the cases group was significantly higher than that in the control group, none of the trace elements were associated with total bilirubin concentration ($P > 0.05$). In this study, we also found that the concentrations of Co, Fe and Se were associated (or tended to be associated) with the TBARS and GSH concentrations ($P < 0.05$, fg. 1). Importantly, our previous study demonstrated that oxidative is participated in the pathogenesis of VPA-induced liver diseases (Ma *et al.*, 2020). These results confirmed that the concentrations of Fe, Co and Se may contribute to VPA-associated hepatotoxicity by regulating the oxidative stress pathway.

Analysis of the risk factors for VPA-associated hepatotoxicity

In this study, logistic regression analysis was performed to further demonstrate the influence of trace elements on VPA-associated hepatotoxicity. As shown in table 5, the concentration of Fe was positively associated with VPA-associated hepatotoxicity ($P = 0.001$, OR: 2.387, 95% CI: 1.530 – 3.617). Meanwhile, the concentrations of Co ($P = 0.038$, OR: 0.889, 95% CI: 0.735 – 1.071) and Se ($P = 0.001$, OR: 0.813, 95% CI: 0.677 – 0.950) were negatively correlated with VPA-associated hepatotoxicity (Fig. 2). In addition, the concentrations of Cr, Cu, Mn, Mo, Sr and Zn were not correlated with VPA-associated hepatotoxicity ($P > 0.05$).

DISCUSSION

Accumulating evidence indicates that the status of trace elements was associated with the development of alcoholic liver disease (ALD) and NAFLD (Tadokoro *et al.*, 2023; Tinkov *et al.*, 2024). However, due to the complicated compositions of trace elements, biomarkers for the susceptibility to VPA-associated hepatotoxicity remain elusive. In this study, we demonstrate that concentrations of Fe, Co and Se may contribute to VPA-associated hepatotoxicity via the oxidative stress pathway (Fig. 2).

Iron (Fe) is an necessary element for the growth and metabolic processes of living organisms (Khan *et al.*, 2020). Perturbation of iron homeostasis may produce oxidative stress conditions and result in a series of related disease (such as immune response, carcinogenesis and liver diseases) (Li *et al.*, 2015; Forcados *et al.*, 2017; Khan *et al.*, 2020). Importantly, our previous study demonstrated that ferroptosis (characterized by iron overload and oxidative stress accumulation) promotes VPA-induced hepatic steatosis in mice and HepG2 cells (Yan *et al.*, 2024). However, there are limited data about the influence of iron status on VPA-induced hepatotoxicity in epileptic patients. In this study, epileptic patients in the cases group exhibited significantly increased Fe concentration ($P < 0.001$, table 3). Meanwhile, the serum Fe concentration was negatively associated with the concentration of GSH and positively correlated with TBARS concentration ($P < 0.05$, Fig. 1). Moreover, logistic regression analysis indicated that Fe was associated with VPA-induced hepatotoxicity ($P = 0.001$, OR: 2.387, 95% CI, 1.530 - 3.617, table 5). These results suggest that iron concentration may contribute to VPA-associated via oxidative stress pathway.

Cobalt (Co), one of the essential trace elements, is widely used in the biomedical and petrochemical industries (Zhao *et al.*, 2023). Co plays a biologically integral role as a metal constituent of vitamin B_{12}, and participates in multiple biological processes (including DNA synthesis and regulation, red blood cell formation, methyl transfer, etc.) (Giedyk *et al.*, 2015). Tinkov and colleagues confirmed that Co has a beneficial effect on the response to NAFLD (Tinkov *et al.*, 2024). Meanwhile, cobalt protoporphyrin was confirmed to ameliorate cholestatic liver disease via heme oxygenase-1 induction (Kim *et al.*, 2021). However, the influence of Co on VPA-associated hepatotoxicity has rarely been investigated. In this study, we found that patients in the cases group had lower Co concentrations than that in the control group ($P < 0.05$, table 3). Importantly, multiple regression analysis and linear regression indicated that Co was negatively associated with transaminase activities but positively correlated with glutathione concentration ($P < 0.05$, table 4 and Fig. 1). Moreover, logistic analysis showed that Co has protective effect on valproic acid-associated hepatotoxicity ($P = 0.038$, OR: 0.889, 95% CI, 0.735 – 1.071, table 5). These results and findings suggest that Co may protect against the development of VPA-associated hepatotoxicity.

Selenium (Se) is an essential micronutrient that have pleiotropic effects, including antioxidant, anti-inflammatory and hormone production (Rayman, 2012). Se and its compounds exhibited key antioxidant activities and play a vital role in the protection against obesity, cancer and liver diseases (Lin *et al.*, 2022; Ali *et al.*, 2024; Bizerea-Moga *et al.*, 2024). Importantly, previous study revealed that patients with VPA treatment showed a higher level of oxidative stress and a lower serum Se concentration (Lin *et al.*, 2022). Similarly, in the current study, we also noticed that serum Se concentration was positively associated with the GSH concentration ($r = 0.3034$, $P = 0.0003$, Fig. 1) but tended to negatively correlate with TBARS concentration in epileptic patients with VPA therapy ($r = -0.1550$, $P = 0.0704$, Fig. 1). Meanwhile, patients in the cases group exhibited a significant lower Se concentration that that in the control group ($P < 0.001$, table 3). Moreover, logistic analysis showed that the serum Se has protective effect on VPA-associated hepatotoxicity ($P = 0.038$, OR: 0.813, 95% CI, 0.677 – 0.950, table 5). These results and findings demonstrated that Se may protect against the pathogenesis of VPA-associated hepatotoxicity by regulating redox state.

Interestingly, although the serum Zinc (Zn) concentration did not differ between the two groups ($P = 0.061$, table 3), the Zn level tended to be negatively associated with serum ALT activity ($P = 0.079$, table 4) and VPA-associated hepatotoxicity ($P = 0.068$, OR: 1.040, 95% CI :0.917–1.276, table 5). Crucially, ahangar and colleagues demonstrated that Zn supplementation may ameliorate VPA-induced hepatotoxicity by regulating oxidative stress levels (Ahangar *et al.*, 2017). Meanwhile, another study confirmed that zinc level in serum and hair were reduced at the 3rd and 6th months of VPA treatment in pediatric patients (Yilmaz *et al.*, 2009). For these reasons, serum Zn concentration may also serve as a potential predictor for VPA-associated hepatotoxicity. Further studies are needed to elucidate the role of Zn in VPA-associated hepatotoxicity.

Finally, the limitations of this study should also be discussed. First, the main sources of trace elements for humans are food and the living environment. However, due to the different daily diets and environmental conditions of each patient, the changes of trace element levels may be not completely influenced by VPA treatment. Second, in this study, 25 patients (approximately 18.2% of the total patients) received concomitant drug therapy. Although concomitant drugs are prescribed only when the patients suffered a high frequency of recurrence of unprovoked seizures, the effects of concomitant drugs on trace element and hepatotoxicity cannot be completely excluded. Third,

considering the overlap of trace elements concentrations between both groups, the status of trace elements did not explain all hepatotoxic cases with VPA-associated hepatotoxicity, further study and clinical validation are required to verify these findings and explore their implications for personalized medicine in epilepsy treatment.

CONCLUSION

This study revealed that patients with VPA-induced hepatotoxicity presented significantly altered concentrations of trace elements (Fe, Co and Se). Importantly, the concentrations of certain trace elements (Fe, Co and Se) are associated with serum transaminase activities and may contribute to (at least partly) the pathogenesis of VPA-associated hepatotoxicity via the oxidative stress pathway. The present study provides a novel insight into VPA-induced hepatotoxicity and may be helpful for preventing hepatotoxicity in patients receiving VPA treatment. We emphasize the importance of monitoring of Fe, Co and Se concentrations in patients receiving long-term VPA therapy.

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SUPPLEMENTARY DATA

Table S1: Demographic data of patients (with valproic acid monotherapy) in the hepatotoxicity and control groups

Demographic characteristics	Cases	Control	*P* value
Number of patients	36 (32.1 %)	76 (67.9 %)	N/A
Age (yrs)	5.04 ± 3.28	4.66 ± 2.88	0.521
Height (cm)	109.00 ± 37.26	102.58 ± 25.18	0.353
Body weight (kg)	25.14 ± 9.92	21.42 ± 10.01	0.068
BMI (kg/m^2)	20.25 ± 5.51	19.60 ± 6.73	0.617
TP (g/L)	68.56 ± 8.08	66.89 ± 5.17	0.262
ALB (g/L)	42.54 ± 8.73	42.73 ± 3.71	0.897
TBiL (μmol/L)	9.35 ± 6.88	6.84 ± 2.48	0.040
GGT (U/L)	47.58 ± 37.45	17.92 ± 13.69	＜0.001
ALP (U/L)	188.28 ± 131.26	185.95 ± 59.95	0.920
ALT (U/L)	85.00 ± 45.27	12.82 ± 7.09	＜0.001
AST (U/L)	98.97 ± 67.48	25.49 ± 6.60	＜0.001
GSH (μmol/L)	33.52 ± 4.46	48.10 ± 3.76	＜0.001
TBARS (μmol/L)	2.31 ± 0.44	2.07 ± 0.36	0.006

The bolded data indicated $P < 0.05$

Table S2: Comparison of serum trace elements in hepatotoxicity and control groups (with valproic acid monotherapy)

Trace elements	Cases (n = 36)	Control (n = 76)	*P* value
Co (ng/mL)	0.66 ± 0.12	0.72 ± 0.13	0.041
Cr (ng/mL)	1.58 ± 0.45	1.69 ± 0.54	0.275
Cu (μg/mL)	1.26 ± 0.21	1.25 ± 0.17	0.722
Fe (μg/mL)	1.25 ± 0.13	1.16 ± 0.12	＜0.001
Mn (ng/mL)	1.95 ± 0.35	1.93 ± 0.26	0.842
Mo (ng/mL)	1.56 ± 0.23	1.62 ± 0.18	0.185
Se (ng/mL)	96.89 ± 11.17	106.92 ± 10.31	＜0.001
Sr (ng/mL)	38.61 ± 10.53	40.03 ± 8.91	0.457
Zn (μg/mL)	0.84 ± 0.10	0.87 ± 0.12	0.180

The bolded data indicated $P < 0.05$