Interdiscip Toxicol. 2020; **Vol. 13**(2): 65–71.
doi: 10.2478/intox-2020-0008

sciendo

interdisciplinary toxicology

ORIGINAL ARTICLE

Effects of long term antiepileptic therapy on serum trace element levels in epileptic children

Ashish Kumar KAKKAR [1], Sheffali GULATI [2], Sudhir Chandra SARANGI [3], Yogendra Kumar GUPTA [3]

[1] Department of Pharmacology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
[2] Child Neurology Division, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India
[3] Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India

ITX130220A03 • Received: 17 October 2017 • Accepted: 17 March 2018

ABSTRACT

Conventional anticonvulsants including valproate (VPA) and phenytoin (PHT) have been shown to influence the trace element homeostasis in patients with epilepsy. Even though the newer agents such as levetiracetam (LEV) are considered to have a better tolerability profile, given the lack of long-term data this confidence is somewhat guarded. In this study, we evaluated the trace element profiles of epileptic children receiving conventional agents *i.e.*, PHT and VPA and newer agent – LEV and compared them with healthy controls. The study groups based on drug treatment were as follows: Group-I (n=35) children received PHT, Group-II (n=30) received VPA, Group-III (n=27) were treated with VPA plus LEV while Group-IV (n=28) included healthy children. Serum levels of zinc, copper, selenium, strontium, magnesium, manganese, and iron were estimated using inductively coupled plasma-atomic emission spectrometry (ICP-AES). As compared to healthy controls, monotherapy with phenytoin was associated with increased serum levels of copper (1568.8 vs. 1053.6 ng/ml; p=0.002) as well as strontium (37.0 vs. 30.7 ng/ml; p=0.014). On the contrary, epileptic children treated with valproate monotherapy had decreased serum selenium (67.0 vs. 84.7 ng/ml; p=0.02) and zinc (1010.5 vs. 1242.9 ng/ml; p=0.003) concentrations. However, in the valproate plus levetiracetam therapy group, no significant alterations in the trace element status were observed. Our findings suggest a potential association between treatment with PHT and VPA and trace element changes in children diagnosed with epilepsy. However, newer agent LEV when used with VPA was not associated with these alterations. Further prospective studies are warranted to confirm our findings and to assess the impact of drug treatment on trace element homeostasis in epileptic children.

KEY WORDS: antiepileptic drugs; trace element status; phenytoin; valproate; levetiracetam

Introduction

Epilepsy and epileptic syndromes are among the most common neurological conditions in childhood and adolescence. Based on epidemiological data, the overall prevalence of childhood onset epilepsy is upto 5.5 per 1000 in developed countries and upto 44 per 1000 in developing countries (Camfield and Camfield, 2015). Higher incidence of childhood epilepsy in developing world is possibly linked to greater incidence of trauma and central nervous system (CNS) infections (De Bittencourt *et al.*, 1996; Peter & Carol, 2006). Conventional antiepileptic drugs (AEDs) are generally considered as first line drugs as they are more affordable and their long term safety profile is well characterized. The newer AEDs are indicated when patients fail to respond to or are intolerant to conventional drugs (Indian Epilepsy Society, 2008; Roy & Das, 2013). However, pharmacoepidemiological studies have indicated a progressive increase in prescription of newer AEDs to children and adolescents (Ackers *et al.*, 2007; Kwong *et al.*, 2012; Nicholas *et al.*, 2012).

Trace elements are critical building blocks in various organ systems including central nervous system(CNS) and several of them viz. zinc, copper and selenium are essential elements of enzymes involved in various metabolic processes (Ashrafi *et al.*, 2007; Frederickson *et al.*, 2012; Hunt, 1980). Alteration in the levels of trace elements and consequent deficient functioning of antioxidant defense mechanisms have been implicated in neuronal excitotoxicity, seizure recurrence as well as

Correspondence address:
Ashish Kumar Kakkar, MD.
Department of Pharmacology,
Postgraduate Institute of Medical Education and Research,
Chandigarh, India
E-MAIL: drashishkakkar@gmail.com

intractability (Ashrafi *et al.*, 2007; Savaskan *et al.*, 2003; Seven *et al.*, 2013). Antiepileptic drugs can possibly alter the levels of trace elements, however, the relationship has been controversial and never convincingly documented (Nazıroğlu & Mürekli, 2013). Several adverse reactions associated with AED therapy have also been attributed to alteration of these crucial elements (Armutcu *et al.*, 2004; Hurd *et al.*, 1984; Palm *et al.*, 1986; Yuen *et al.*, 1988). Studies of copper, manganese, selenium, and zinc, status in patients receiving conventional antiepileptics viz. valproate(VPA), phenytoin (PHT), carbamazepine (CBZ), and phenobarbitone have demonstrated considerable alterations in trace element levels, however, the results have been contradictory and uncertainty prevails (Armutcu *et al.*, 2004; Castilla-Guerra *et al.*, 2006; Hamed *et al.*, 2004; Kürekçi *et al.*, 1995; Kuzuya *et al.*, 1993; Suzuki *et al.*, 1992; Verrotti *et al.*, 2002). Few studies till date have evaluated the impact of newer antiepileptic drug, levetiracetam on serum trace element status in pediatric epilepsy patients. Therefore, this study was conducted to evaluate the level of a panel of nine serum trace elements namely zinc , copper , selenium , magnesium , manganese , iron, strontium (Sr), lead (Pb) and cadmium (Cd)] in epileptic children receiving conventional (phenytoin/ valproate) and newer AEDs (levetiracetam as an add-on therapy) and compare them with healthy controls.

Methods

Study settings and participants

This was a cross-sectional study that included children diagnosed with epilepsy, of either sex, aged 6–16 years, and receiving antiepileptic drug therapy for a minimum of six months, from child neurology clinic at a tertiary care institute – All India Institute of Medical Sciences – Delhi, located in northern part of India. The drugs considered were VPA and PHT – as monotherapy, and combination therapy with valproate plus levetiracetam (VPA+LEV). Subjects unwilling to provide informed consent/ assent, receiving other medications or dietary/trace element supplements, having signs of malnutrition, dietary restrictions due to a concomitant diagnosis, abnormal neurologic examination findings, intellectual disability and progressive brain disease or serious co-morbidities including severe cardiac, renal, pulmonary, hepatic or other systemic disease or cancer were excluded.

Apparently healthy children who were first degree cousins of study patients were recruited as controls. This was done to correct for socioeconomic and geographical differences that might influence the trace element levels. The Institute Ethics Committee, AIIMS approved the study protocol. The details of the study were explained prior to enrollment, with the consent form signed by all parents and assent taken from pediatric patients as well as healthy controls. The study was conducted in accordance with Declaration of Helsinki and Good Clinical Practice(ICH-GCP) guidelines.

For children with epilepsy the following information were obtained: complete medical history and clinical examination, age, gender, age of onset of seizures, prescribed antiepileptic drugs and compliance to therapy. Geographical, sociodemographic background; dietary habits; use of dietary supplements; results of biochemical and radiological investigations were also recorded. For each child, height and weight were recorded. In addition, they were evaluated for the clinical signs of malnutrition. All the participant children were seizure free for a minimum of 24 hours in the period preceding the sampling of blood. The latter was done as per the guidelines of International Union of Pure and Applied Chemistry (Cornelis *et al.*, 1996).

Estimation of trace element levels

To begin, dissolution of study samples was performed as described previously (Sarangi *et al.* 2014) using microwave assisted digestion system Following the digestion procedure clear solutions were obtained that were analyzed using Inductively Coupled PlasmaAtomic Emission Spectrometry (ICP-AES) to determine serum levels of zinc, copper, selenium , manganese , magnesium , iron , strontium , lead and cadmium. (The study samples were analyzed using equipment JY 2000-2 from HORIBA Jobin Yvon, France).

A multi-element standard solution that contained 1000 mg/L of 23 elements in 1 mol/L nitric acid was used as reference standard (Merck Chemicals, Germany). Selenium estimation was carried out using selenium standard (Sigma Chemicals, USA), separately along with concomitant metal analyzer. ICP-AES as described above and fitted with a quartz spray chamber and a cross flow nebulizer was employed throughout the estimation with the following conditions: forward power = 1200W; nebulizer flow rate = 0.76 L per min dual detector and sweep/ reading = 3, replicates = 3, dwell time = 5 seconds, and an integration time = 10 seconds. Wavelengths for each trace element were chosen from a pre-defined set using the ICP proprietary software v. 5.2. Background correction was done using a blank solution. The same batch of samples were run in duplicate for precision. The limit of detection, as detailed in the instrument manual was used for study calculations. For calibration curves, the standard mixture solution was diluted stepwise with five percent nitric acid in the concentration range of 5 to 100 parts per billion. The calibration curves had a good linearity with a correlation coefficient of ≥0.993.

Statistical analysis

Sample size was estimated using G*Power v. 3.1.3 statistical software. The mean serum levels of zinc in epileptic children receiving 6 months of valproate therapy formed the basis for calculation (Yilmaz *et al.*, 2009). For alpha equal to 0.05 and power of 90%, a sample size of 26 children per group was required for the study.

Data were analyzed using software SPSS v.16 – (Statistical Package for the Social Sciences Chicago, IL, USA). Distribution of study data was checked using

the Shapiro-Wilks method. Comparisons between study groups were made for variables following normal distribution using analysis of variance (ANOVA) test, while Kruskal Wallis test was used for variables not demonstrating a normal distribution. Appropriate post hoc analysis were carried out for statistically significant differences. Categorical variables were analyzed using Chi square test. Since the trace element levels followed a non-normal distribution, statistical comparisons were made using median and range and the level of significance was considered as <0.05.

Results

Study subjects and demographics

A total of 126 epileptic children and 36 healthy controls were screened for enrolment into the study. Out of them 92 patients and 28 healthy controls were found eligible and agreed to participate in the study (Figure 1).

Based on the antiepileptic drug therapy received by these children, they were grouped as:

- Group I: Phenytoin monotherapy (PHT, n=35);
- Group II: Valproate monotherapy (VPA, n=30);
- Group III: Levetiracetam add on therapy to valproate (LEV+VPA, n=27);
- Group IV: Healthy controls (n=28).

There were no significant differences between the four study groups with respect to age, sex distribution, age in years at onset of epilepsy, seizure-free rate and place of residence (rural/urban) (Table 1). The mean duration of therapy with study drugs was 17.97±9.26 months in phenytoin treated group, 19.20±10.23 months in valproate treated group and 12.85±6.13 months in children receiving levetiracetam treatment. The latter group also received valproate for a mean duration of 22.44±12.59 months. Analysis of Body Mass Index (BMI) of study participants in different study groups revealed a significantly higher BMI in children treated with valproate monotherapy (p<0.001) when compared with other patients as well as healthy controls (Table 1).

Serum trace element levels

The Limits of Detection (LoD) for all trace elements reported in the study were below 1 µg/L (except lead). The serum trace elements levels for children with epilepsy in the three study groups and controls are summarized in Table 2.

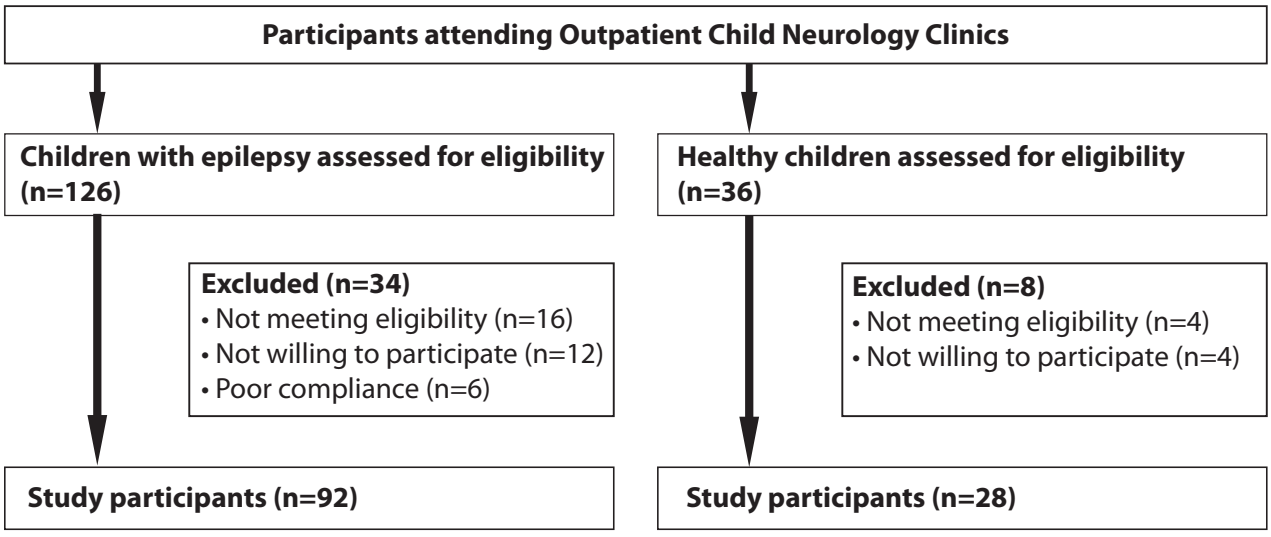

Figure 1. Flow of participants into the study.

Table 1. Demographic and clinical characteristics of study participants.

Parameter	Group I (PHT) (n=35)	Group II (VPA) (n=30)	Group III (LEV+VPA) (n=27)	Group IV (Controls) (n=28)	P value
Age(yrs)	9.43±2.48	9.9±2.74	8.78±2.75	10.02±2.61	0.24
Gender (M/F)	25/10	25/5	22/5	19/9	0.43
BMI (kg/m^2)	15.03±2.69	18.58±3.82	16.28±3.43	15.38±2.27	<0.001
Age at onset (yrs)	6.96±3.1	6.16±3.09	5.1±3.42	–	0.08
Seizure free rate (%)	71.4	60	48.1	–	0.18
Residence (Urban/Rural)	21/14	20/10	16/11	12/16	0.3

Data expressed as mean±S.D except when indicated; PHT=Phenytoin, VPA=Valproate, LEV= Levetiracetam, M=Male, F=Female, BMI= Body mass index.

Table 2. Comparison of trace elements levels among children receiving conventional and new AEDs

Elements	Group I (PHT)		Group II (VPA)		Group III (LEV+VPA)		Group IV (Control)		*p*-value (overall)	*p*-value (I vs. IV)	*p*-value (II vs. IV)	*p*-value (III vs. IV)
	Mean±SD	Median and Range	Mean±SD	Median and Range	Mean±SD	Median and Range	Mean±SD	Median and Range				
Zn	1332.9±638.1	1135.5 (537.7, 2992.2)	937.7±246.2	1010.5 (465.1, 1464.5)	1066.8±403.9	1067 (460.9, 2258.4)	1354.8±531	1242.9 (644.5, 2686.6)	0.014	0.5	0.003	0.05
Cu	1451.3±495.8	1568.8 (603.8, 2197.0)	1111.2±520.2	1100.1 (416.3, 2278.0)	1317.7±527.1	1321.2 (565.3, 1954.4)	1092.1±365.1	1053.6 (575.6, 1886.0)	0.007	0.002	0.97	0.12
Se	78.2±21.3	76.2 (36.7, 126.4)	67.9±22.7	67.0 (29.6,118.6)	81.6±21.2	78.1 (48.9, 121.9)	87.3±30.3	84.7 (35.9, 145.8)	0.04	0.25	0.02	0.52
Mn	1.6±0.6	1.5 (0.5, 3.0)	2.0±0.7	2.1 (0.5, 3.3)	2.0±0.7	2.04 (0.7, 3.61)	1.9±0.9	1.9 (0.4, 3.5)	0.03	0.15	0.29	0.43
Mg (mg/L)	17.7±4.0	18.0 (9.3, 28.4)	19.4±4.6	19.8 (12.7, 31.3)	19.1±3.5	18.4 (13.7, 27.5)	18.2±2.7	18.2 (14.1, 23.7)	0.31	0.53	0.29	0.47
Fe (mg/L)	1.6±0.5	1.5 (1.1, 2.9)	1.7±0.4	1.7 (1.1, 2.6)	1.6±0.4	1.6 (1.1, 2.3)	1.5±0.4	1.4 (1.1, 2.5)	0.10	0.95	0.06	0.27
Sr	39.5±10.6	37.0 (21.9, 75.7)	28.3±11.0	25.0 (14.9, 55.9)	25.9±11.4	24.1 (11.1, 51.4)	31.9±15.1	30.7 (11.3, 65.7)	<0.001	0.014	0.53	0.11

PHT-Phenytoin , VPA-Valproic acid, LEV- Levetiracetam, Zn- Zinc, Cu- Copper, Se- Selenium, Mn- Manganese, Mg- Magnesium, Fe- Iron, Sr- Strontium. All levels expressed as (ng/ml) except when indicated.

Serum zinc levels in the control group and in the phenytoin treated group were comparable. However, when compared to control group, the median serum zinc levels in the valproate treatment group were significantly lower. The serum levels of copper in phenytoin treated participants were significantly higher as compared to the children in the control group (p=0.002). Among the valproate treated children i.e. VPA and VPA+LEV treatment groups, copper levels were not significantly different from the healthy control group (Figure 2).

Serum selenium concentrations were unaltered among the PHT and VPA+LEV groups in comparison to controls, while the VPA monotherapy group exhibited significantly lowered selenium levels (p=0.02) (Table 2). No differences were observed in the levels of manganese, magnesium and iron in all the patient groups in comparison to the controls. Serum strontium levels in the phenytoin treated subjects were found to be higher (p=0.014) as compared to controls. However, no alterations were observed in the strontium levels in the VPA and VPA+LEV groups (Figure 2).

Toxic trace elements, lead and cadmium were below the limit of detection in most study participants. Cadmium could be detected in only two patients and one healthy control. Lack of sufficient number of valid cases precluded any statistical comparisons. However, lead was detected in all the patient groups but not among healthy controls. The differences in the mean serum lead levels in the patient groups were not significant. The correlation between duration of treatment and serum levels of trace elements was not found to be significant.

Discussion

Several studies in the past have demonstrated abnormalities in serum trace elements concentrations in epileptic patients, adults as well as children (Ashraf *et al.*, 1995; Hamed *et al.*, 2004; Sarangi *et al.*, 2014; Verrotti *et al.*, 2002; Wojciak *et al.*, 2013) and significant body of data suggests the role of trace elements in the pathophysiology of epilepsy (Ashrafi *et al.*, 2007; Dudek, 2001; Hamed *et al.*, 2004; Ikeda, 2001; Smith & Bone, 1982). More recent reports have even linked alterations in level of trace elements especially selenium and zinc with treatment response in epilepsy (Ashrafi *et al.*, 2007; Saad *et al.*, 2014; Seven *et al.*, 2013). As noted above data regarding effects of AED therapy on trace element status has been contradictory. There is limited data available evaluating the effect of newer AEDs like levetiracetam on trace element levels and the impact of classic AEDs on trace element levels other than those of zinc, copper and selenium in children with epilepsy receiving long term therapy. Due to the unavailability of standard reference ranges for trace element levels in Indian children, serum levels of these elements in study patients were compared with that of healthy children taken as controls. While alterations were noted in trace element levels in the AED therapy groups, there were important differences in between the study groups. Children treated with valproate alone had significantly higher BMI when compared with other patients as well

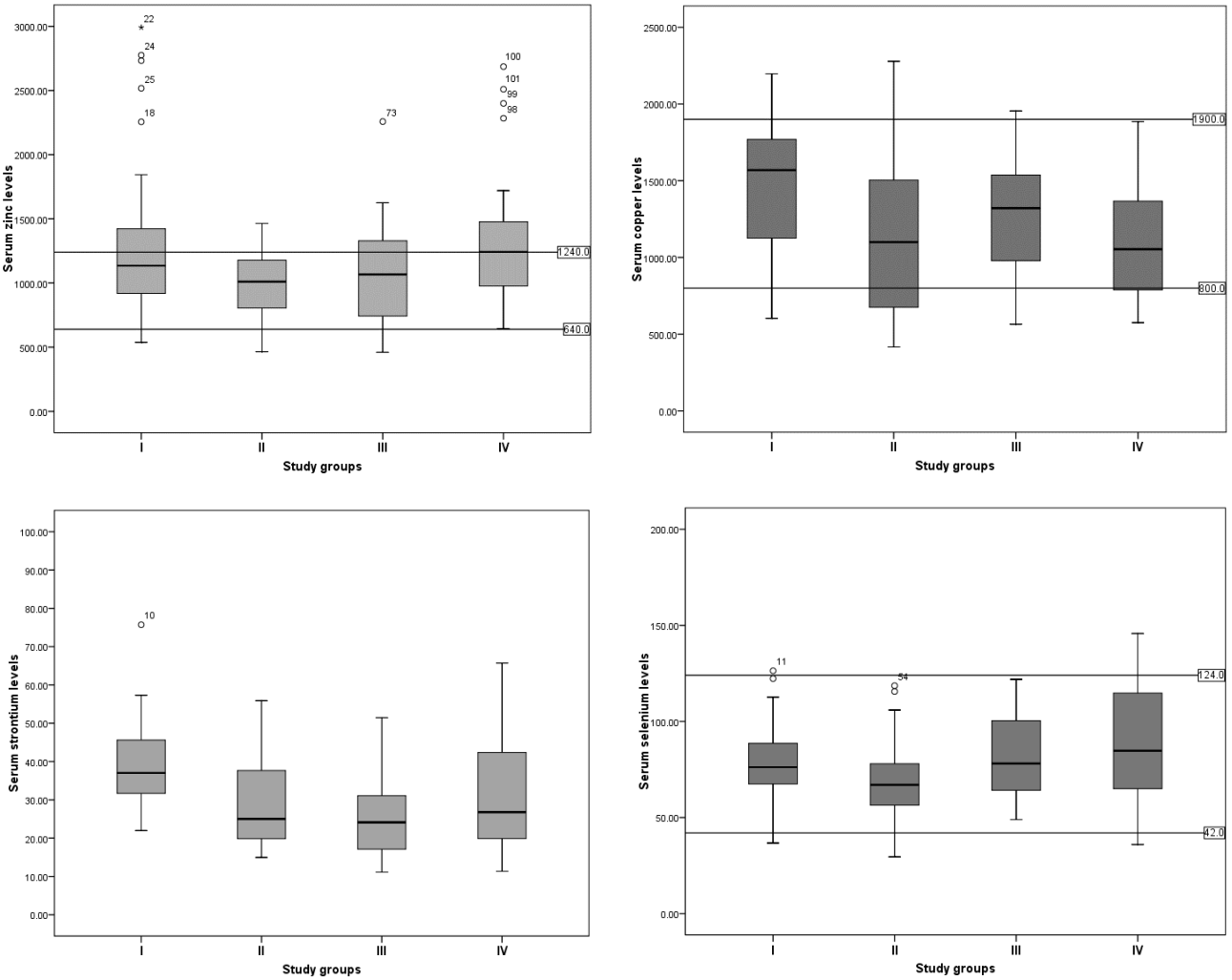

Figure 2. Serum levels of (a) zinc (µg/l) (b) copper (µg/l) (c) strontium (µg/l) (d) selenium (µg/l) among the patient and control groups. (Study groups; Group I=PHT, Group II=VPA, Group III=VPA+LEV, Group IV=Controls).

as healthy controls. This finding was expected since weight gain is a common adverse consequence linked with valproate therapy. On the contrary, both phenytoin and levetiracetam have been classified as weight neutral AEDs (Ben-Menachem, 2007; Sarangi *et al.*, 2016). In support, no significant differences were noted in the BMI of phenytoin treated children when compared to the control group. Similarly, the BMI of children treated with combination of VPA and LEV was also not significantly different from that of controls. A study by Gelisse *et al.* has even reported significant weight loss associated with levetiracetam treatment (Gelisse *et al.*, 2008).

In the present study serum zinc levels in the valproate treatment group were found to be significantly lower when compared to the healthy controls. Our results are in agreement with several other studies that have reported decreased serum zinc concentrations in adult (Kuzuya *et al.*, 1993; Steidl *et al.*, 1987; Suzuki *et al.*, 1992) as well as pediatric (Armutcu *et al.*, 2004; Deniz *et al.*, 2008; Yilmaz *et al.*, 2009) patients treated with VPA. On the contrary, few studies (Doneray *et al.*, 2012; Kaji *et al.*, 1992; Verrotti *et al.*, 2002) have failed to demonstrate any significant effect of VPA administration on serum zinc levels in children with epilepsy. The mechanisms behind reduced serum zinc levels in patients treated with VPA have not been established although it has been postulated that valproate can bind zinc, thus preventing the inhibition of glutamic acid decarboxylase (GAD) by zinc and resulting in an increased level of gamma amino butyric acid (GABA) (Hurd *et al.*, 1981). Other possible mechanisms include reduction of the total zinc concentration via binding zinc and causing its redistribution in tissues and inducing metallothionein synthesis in liver (Keen *et al.*, 1989). Several signs of zinc deficiency such as GI disturbances, taste alterations, pancreatitis, hepatotoxicity, hair loss, stupor, lethargy, tremor and anorexia resemble adverse effects reported with VPA therapy (Lerman-Sagie *et al.*, 1987). In addition, reported association between maternal valproate intake and neural tube defects in newborns could be possibly ascribed to zinc deficiency since zinc deprivation has been shown to be teratogenic in animals (Robert *et al.*, 1984). In contrast, in children receiving levetiracetam add on therapy, serum zinc levels were maintained despite concurrent treatment with VPA.

No significant differences were noted between serum copper levels of epileptic children receiving VPA monotherapy or VPA+LEV therapy when compared to healthy controls. In epilepsy patients treated with VPA, all kinds of alterations in serum copper levels – increase, decrease, and no change, have been previously reported (Doneray *et al.*, 2012; Kaji *et al.* 1992; Kürekçi *et al.*, 1995; Kuzuya *et al.*, 1993; Verrotti *et al.*, 2002). Our results are in agreement with those of Verrotti *et al.*, Kurekci *et al.*, Smith and Bone and Hamed *et al.* Experimental studies in rodents have reported VPA induced increased biliary flow and enhanced biliary excretion of copper as possible causes for decrease in serum copper levels (Kuzuya *et al.*, 2002). However, it has been argued that chronic treatment with VPA despite increasing biliary excretion of copper, may not induce copper deficiency in epileptic patients and that specific oxidase activity of ceruloplasmin may be a more useful biomarker of copper status in such individuals (Tutor-Crespo *et al.*, 2003). A significant increase in serum copper levels in epileptic children treated with phenytoin was observed as compared to healthy controls. An increase in the liver ceruloplasmin synthesis through enzyme-induction by phenytoin has been suggested as a possible mechanism (Palm *et al.*, 1986; Tutor *et al.*, 1982).

Serum selenium levels were reduced in valproate monotherapy group when compared to healthy children but not in the PHT and VPA plusLEV treated children. Few studies in the past have reported unchanged serum selenium levels in patients receiving AED treatment (Kürekçi *et al.*, 1995; Verrotti *et al.*, 2002). However, Hurd *et al.* reported reduced plasma selenium levels in rats treated with VPA and replicated these results in epilepsy patients as well (Hurd *et al.*, 1984). They proposed that valproate associated hepatotoxicity occurring in children could be due to decreased selenium concentrations and consequently reduced glutathione peroxidase (GSH-Px) activity. In our study VPA monotherapy was associated with reduced selenium levels, however none of the participants reported hepatotoxicity.

Strontium levels were significantly higher in the PHT treated children as compared to controls. It has been postulated that since strontium accumulates significantlyin bone, it can interfere with the normal bone development when present in high concentrations. Several studies have shown strontium to be associated with osteomalacia (Cohen-Solal, 2002; Oste *et al.*, 2005). Experimental studies in rats have demonstrated defective mineralization reflected by a decreased bone mineral density on exposure to high doses of strontium (Morohashi *et al.*, 1994). Phenytoin has been associated with osteomalacia as well as osteoporosis. The mechanisms for adverse consequences on bone health are unclear and several plausible mechanisms have been proposed (Pack *et al.*, 2004). Our results demonstrate increased strontium levels associated with phenytoin therapy which may contribute to the bone disease seen with long term phenytoin therapy.

The use of levetiracetam as an add-on treatment for epilepsy in children in our study is in sync with routine clinical practice at the time study was conducted. Treatment with LEV was not associated with any significant trace element alterations in epileptic children as compared to the control group. Possible mechanisms of alteration of element levels include enhanced absorption of metals from gut and/or their reduced elimination from body. LEV by virtue of having minimal metabolism, minimal protein binding, lack of dependence on and interaction with CYP P450 system is less likely to affect trace element status on account of its pharmacokinetics (Patsalos, 2000).

Our study has few limitations including a cross sectional design and lack of previous data on various trace elements in epileptic children which precluded accurate sample size calculation. Precise clinical interpretation of our study results depends, to a large extent, on the use of reference intervals. For various reasons including relatively smaller blood volumes and parental reluctance to allow blood to be drawn from healthy children, there has been a paucity of data for determining trace element reference intervals in children. Serum levels of trace elements across populations may vary widely on account of nutritional factors, industrial and environmental exposure, geographical differences and factors affecting soil and water conditions. Since these crucial elements play a vital role in child health, any unwanted alterations with AED therapy can not only affect the treatment response but may also contribute to significant adverse effects. Well-designed large scale prospective studies comparing levels of the trace elements before and after initiation of treatment in children with epilepsy are needed to provide conclusive evidence of impact of AED therapy on trace element status.

Acknowledgements

We hereby acknowledge the assistance provided by Dr. Amita Srivastava and Dr. G Kumar at AIIMS with the analysis of trace elements using ICP-AES.

REFERENCES

Ackers R, Murray ML, Besag FMC, Wong ICK. (2007). Prioritizing children's medicines for research: a pharmacoepidemiological study of antiepileptic drugs. *Br J Clin Pharmacol* **63**: 689–97.

Armutcu F, Ozerol E, Gurel A, Kanter M, Vural H, Yakinci C., Akyol O. (2004). Effect of long-term therapy with sodium valproate on nail and serum trace element status in epileptic children. *Biol Trace Elem Res* **102**(1–3):1–10.

Ashraf W, Jaffar M, Mohammed D, Iqbal J. (1995) Utilization of scalp hair for evaluating epilepsy in male and female groups of the Pakistan population. *Sci Total Environ* **164**: 69–73.

Ashrafi MR, Shabanian R, Abbaskhanian A, Nasirian A, Ghofrani M, Mohammadi M, Zamani GR, Kayhanidoost Z, Ebrahimi S, Pourpak Z. (2007). Selenium and intractable epilepsy: is there any correlation? *Pediatr Neurol* **36**: 25–9.

Ben-Menachem E. (2007). Weight issues for people with epilepsy—a review. *Epilepsia* **48**: 42–45.

Camfield P, Camfield C. (2015). Incidence, prevalence and aetiology of seizures and epilepsy in children. *Epileptic Disorders* **17**(2): 117–123.

Castilla-Guerra L, del Carmen Fernández-Moreno M, López-Chozas JM, Fernández-Bolaños R. (2006). Electrolytes disturbances and seizures. *Epilepsia* **47**: 1990–8.

Cohen-Solal M. (2002). Strontium overload and toxicity: impact on renal osteodystrophy. *Nephrol Dial Transplant* **17**(2): 30–4.

Cornelis R, Heinzow B, Herber RF, Christensen JM, Poulsen OM, Sabbioni E, Templeton DM, Thomassen Y, Vahter M, Vesterberg O. (1996). Sample collection guidelines for trace elements in blood and urine. IUPAC Commission of Toxicology. *J Trace Elem Med Biol* **10**: 103–27.

De Bittencourt PR, Adamolekum B, Bharucha N, Carpio A, Cossío OH, Danesi MA, Dumas M, Meinardi H,Ordinario A, Senanayake N, Shakir R, Sotelo J. (1996). Epilepsy in the tropics: I. Epidemiology, socioeconomic risk factors, and etiology. *Epilepsia* **37**: 1121–7.

Doneray H, Kara IS, Karakoc A, Tan H, Orbak Z. (2012). Serum thyroid hormone profile and trace elements in children receiving valproic acid therapy: A longitudinal and controlled study. *J Trace Elem Med Biol* **26**: 243–7.

Dudek FE. (2001). Zinc and Epileptogenesis. *Epilepsy Curr* **1**: 66–70.

Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB. (2000). Importance of zinc in the central nervous system: the zinc-containing neuron. *J Nutr* **130**(5S): 1471S–83S.

Gelisse P, Juntas-Morales R, Genton P, Hillaire-Buys D, Diaz O, Coubes P, Crespel A. (2008). Dramatic weight loss with levetiracetam. *Epilepsia* **49**: 308–315.

Hamed SA, Abdellah MM, El-Melegy N (2004). Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients. *J Pharmacol Sci* **96**: 465–73.

Hunt DM. (1980). Copper and neurological function. *Ciba Found Symp* **79**: 247–66.

Hurd RW, Van Rinsvelt HA, Wilder BJ, Karas B, Maenhaut W, De Reu L. (1984). Selenium, zinc, and copper changes with valproic acid: possible relation to drug side effects. *Neurology* **34**: 1393–5.

Hurd RW, Wilder BJ, Street JJ, Sciscent BL. (1981). Zinc binding by valproic acid. *Neuroscience Abstr* **7**: 813.

Ikeda M. (2001). Iron overload without the C282Y mutation in patients with epilepsy. *J Neurol Neurosurg Psychiatr* **70**: 551–3.

Indian Epilepsy Society. 2008. Guidelines for Management of Epilepsy in India (GEMIND). [accessed on 23 Jul 2017] Available from: http://www.ilae.org/visitors/centre/guidelines.cfm

Kaji M, Ito M, Okuno T, Momoi T, Sasaki H, Yamanaka C, Yorifuji T, Mikawa H. (1992). Serum copper and zinc levels in epileptic children with valproate treatment. *Epilepsia* **33**: 555–7.

Keen CL, Peters JM, Hurley LS. (1989). The effect of valproic acid on 65Zn distribution in the pregnant rat. *J Nutr* **119**: 607–11.

Kürekçi AE, Alpay F, Tanindi S, Gökçay E, Ozcan O, Akin R, Işimer A, Sayal A. (1995). Plasma trace element, plasma glutathione peroxidase, and superoxide dismutase levels in epileptic children receiving antiepileptic drug therapy. *Epilepsia* **36**: 600–4.

Kuzuya T, Amioka K, Nabeshima T. (2002). Valproic acid increases biliary copper excretion in the rat. *Epilepsy Res* **51**: 279–85.

Kuzuya T, Hasegawa T, Shimizu K, Nabeshima T. (1993). Effect of anti-epileptic drugs on serum zinc and copper concentrations in epileptic patients. *Int J Clin Pharmacol Ther Toxicol* **31**: 61–5.

Kwong KL, Tsui KW, Wu SP, Yung A, Yau E, Eva F, Ma KC, Cherk S, Liu KT, Cheng WW, Yau MM. (2012). Utilization of antiepileptic drugs in Hong Kong children. *Pediatr Neurol* **46**: 281–6.

Lerman-Sagie T, Statter M, Szabo G, Lerman P. (1987). Effect of valproic acid therapy on zinc metabolism in children with primary epilepsy. *Clin Neuropharmacol* **10**: 80–6.

Morohashi T, Sano T, Yamada S. (1994). Effects of strontium on calcium metabolism in rats. I. A distinction between the pharmacological and toxic doses. *Jpn J Pharmacol* **64**: 155–62.

Nazıroğlu M, Yürekli VA. (2013). Effects of antiepileptic drugs on antioxidant and oxidant molecular pathways: focus on trace elements. *Cell Mol Neurobiol* **33**: 589–99.

Nicholas JM, Ridsdale L, Richardson MP, Ashworth M, Gulliford MC. (2012). Trends in antiepileptic drug utilisation in UK primary care 1993–2008: Cohort study using the General Practice Research Database. *Seizure* **21**: 466–70.

Oste L, Bervoets AR, Behets GJ, Dams G, Marijnissen RL, Geryl H, Lamberts LV, Verberckmoes SC, Van Hoof VO, De Broe ME, D'Haese PC. (2005). Time-evolution and reversibility of strontium-induced osteomalacia in chronic renal failure rats. *Kidney Int* **67**: 920–30.

Pack AM, Gidal B, Vazquez B. (2004). Bone disease associated with antiepileptic drugs. *Cleve Clin J Med* **71**(2): S42–48.

Palm R, Hallmans G, Wahlström G. (1986). Effects of long-term phenytoin treatment on brain weight and zinc and copper metabolism in rats. *Neurochem Pathol* 5: 87–106.

Patsalos PN. (2000). Pharmacokinetic profile of levetiracetam: toward ideal characteristics. *Pharmacol Ther* **85**: 77–85.

Peter RC, Carol S. (2006). Camfield, pediatric epilepsy: An overview, in : *Pediatric Neurology principles and practice (*Swaiman KF) pp. 981-989, Mosby, 4 th edition.

Robert E, Löfkvist E, Mauguiere F. (1984). Valproate and spina bifida. *Lancet* **2**: 1392.

Roy MK, Das D. (2013). Indian Guidelines on Epilepsy. Chapter 116. Section 16: Neurology. Medicine Update, 2013. The Association of Physicians of India. [accessed 21 Jul 2017]. Available from: http://www.apiindia.org/medicine_update_2013/chap116.pdf.

Saad K, Hammad E, Hassan AF, Badry R. (2014). Trace element, oxidant, and antioxidant enzyme values in blood of children with refractory epilepsy. *Int J Neurosci* **124**: 181–6.

Sarangi SC, Tripathi M, Kakkar AK, Gupta YK. (2014). Effect of antiepileptic therapy on trace elements status in Indian population in a tertiary care hospital from northern India: A cross sectional study. *Epilepsy Res* **108**: 917–27.

Sarangi SC, Tripathi M, Kakkar AK, Gupta YK. (2016). Comparison of body composition in persons with epilepsy on conventional & new antiepileptic drugs. *Indian J Med Res* **143**(3): 323–30.

Savaskan NE, Bräuer AU, Kühbacher M, Eyüpoglu IY, Kyriakopoulos A, Ninnemann O, Behne D, Nitsch R. (2003). Selenium deficiency increases susceptibility to glutamate-induced excitotoxicity. *FASEB J* **17**: 112–4.

Seven M, Basaran SY, Cengiz M, Unal S, Yuksel A. (2013). Deficiency of selenium and zinc as a causative factor for idiopathic intractable epilepsy. *Epilepsy Res*. **104**: 35–9.

Smith WG, Bone I. (1982). Copper, zinc and magnesium plasma levels in epilepsy. *J Neurol Neurosurg Psychiatr* **45**: 1072.

Steidl L, Tolde I, Svomová V. (1987). Metabolism of magnesium and zinc in patients treated with antiepileptic drugs and with magnesium lactate. *Magnesium* **6**: 284–95.

Suzuki T, Koizumi J, Moroji T, Shiraishi H, Hori T, Baba A, Kawai N, Tada K. (1992). Effects of long-term anticonvulsant therapy on copper, zinc, and magnesium in hair and serum of epileptics. *Biol Psychiatry* **31**: 571–81.

Tekin D, Taşdemir HA, Saraymen R. (2008). The effects of antiepileptic drugs on serum and hair trace element levels. Ankara Üniversitesi Tıp Fakültesi Mecmuası. **61**(2).

Tutor JC, Fernandez MP, Paz JM. (1982). Serum copper concentration and hepatic enzyme induction during long-term therapy with anticonvulsants. *Clinical Chemistry* **28**: 1367–70.

Tutor-Crespo MJ, Hermida J, Tutor JC (2003). Assessment of copper status in epileptic patients treated with anticonvulsant drugs by measuring the specific oxidase activity of ceruloplasmin. *Epilepsy Res* **56**: 147–53.

Verrotti A, Basciani F, Trotta D, Pomilio MP, Morgese G, Chiarelli F (2002). Serum copper, zinc, selenium, glutathione peroxidase and superoxide dismutase levels in epileptic children before and after 1 year of sodium valproate and carbamazepine therapy. *Epilepsy Res* **48**: 71–5.

Wojciak RW, Mojs E, Stanislawska-Kubiak M, Samborski W. (2013). The serum zinc, copper, iron, and chromium concentrations in epileptic children. *Epilepsy Res* **104**: 40–4.

Yilmaz Y, Tasdemir HA, Paksu MS. (2009). The influence of valproic acid treatment on hair and serum zinc levels and serum biotinidase activity. *Eur J Paediatr Neurol* **13**: 439–43.

Yuen WC, Whiteoak R, Thompson RP. (1988). Zinc concentrations in leucocytes of patients receiving antiepileptic drugs. *J Clin Pathol* **41**: 553–5.