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Effect of antiepileptic therapy on trace elements status in Indian population in a tertiary care hospital from northern India: A cross sectional study

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Summary
Aim: Conventional antiepileptics (AEDs) have been shown to alter the homeostasis of copper, zinc, and selenium in persons with epilepsy (PWE). The effects of newer AEDs on trace elements have not been addressed yet. This cross-sectional study evaluated trace elements and electrolytes status in PWE on conventional and newer AEDs treatment.
Methods: A total of 307 adult persons with epilepsy and 42 healthy controls were recruited. Panels of ten trace elements estimated by inductively coupled plasma-atomic emission spectrometry, electrolytes, liver and renal function status were compared among subjects grouped according to the monotherapy of AEDs and type of conventional and newer AEDs.
Results: Out of the total 307 PWE, 171 were on monotherapy [valproic acid (VPA) ($n=50$), carbamazepine ($n=47$), phenytoin ($n=49$), levetiracetam ($n=21$), lamotrigine ($n=4$)]. AEDs monotherapy groups had no significant difference in the trace element levels, except higher nickel level in levetiracetam group and low iron level in lamotrigine group compared to VPA group. Compared to control [zinc level 698.0 (367.8—3084.4) ng/ml], levetiracetam group had higher zinc [1293.1 (997.7—2419.7) ng/ml, $p<0.0001$], selenium, copper, iron, aluminium,

Abbreviations: AEDs, antiepileptic drugs; ADRs, adverse drug reactions; AIIMS, All India Institute of Medical Sciences; IUPAC, International Union of Pure and Applied Chemistry; ICP-AES, inductively coupled plasma-atomic emission spectrometry; LOD, limit of detection.

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cadmium, cobalt, and nickel levels; similar manganese and lead levels. Other monotherapy groups were having similar metal levels as that of levetiracetam group except nickel, iron, lead, and selenium levels.
Conclusion: Trace element status was significantly altered with both conventional and newer antiepileptic drugs as compared to control; however, there was not much difference in between conventional and new drug treated groups. Prospective studies will address its impact on treatment response and adverse effect profile.
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Introduction

As per Indian Epilepsy Society, there are approximately ten million people in India living with epilepsy and 70—80% of them respond to treatment (Indian Epilepsy Society, 2008; Meinardi et al., 2001; Ngugi et al., 2010). Epilepsy treatment is preferably initiated with conventional antiepileptic drugs (AEDs) as they are less expensive and the side effects are well characterized (Indian Epilepsy Society, 2008; Mac et al., 2007; Roy and Das, 2013). However, the pattern of AEDs use reveal that nearly 50% of the patients are put on one of the newer AEDs either as monotherapy or as an add-on drug in polytherapy (Indian Epilepsy Society, 2008; Krishnan et al., 2003; Mac et al., 2007; Sigamani et al., 2006). There is claim of better tolerability profiles with newer drugs, conversely, some authors have shown that there is no significant difference in frequency and severity of adverse drug reactions (ADRs) between conventional and newer AEDs (Khanna et al., 2009; S et al., 2008). Maximum ADRs have been observed with AED polytherapy.

There is evidence of negative impact of AEDs on the antioxidant status of body including scavenging of free radicals, chelation of ion metals, and trace elements status (Hamed et al., 2004; Kürekçi et al., 1995; Verrotti et al., 2002). Trace elements [e.g., zinc (Zn), selenium (Se), and copper (Cu)] are minor building components in tissues including the nervous system; they act as cofactors for most of the enzymes in the biological system (Ashrafi et al., 2007; Hunt, 1980; Westbrook and Mayer, 1987). Deficiency or excess of some essential trace elements have been associated with several neurological abnormalities including seizures (Ashrafi et al., 2007; Hunt, 1980; Seven et al., 2013; Westbrook and Mayer, 1987). Studies of Cu, manganese (Mn), Se, Zn, and electrolytes status in subjects on conventional antiepileptics [valproic acid (VPA), carbamazepine (CBZ), phenytoin (PHT) and phenobarbitone] have shown significant alteration in element metabolism and free radical scavenging enzyme activities, however, these results are conflicting and inconclusive (Castilla-Guerra et al., 2006; Hamed et al., 2004; Kürekçi et al., 1995; Suzuki et al., 1992; Verrotti et al., 2002). To the best of our knowledge no studies have evaluated the effect of AEDs on aluminium, cadmium, cobalt, nickel, and lead levels, which have association with several neurological disorders including epilepsy (Baydar et al., 2003; Cheong et al., 1999; Cooper and Legare, 1997; Denays et al., 2005; González-Estecha et al., 2011). The effects of newer AEDs on the status of trace elements have not been addressed as yet. Therefore, in this study, we evaluated the level of a panel of ten trace elements [aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn)] and electrolytes [sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and phosphate (PO_4^{3-})]; and liver and renal function status in persons with epilepsy on conventional and newer AEDs.

Methods

Study setting and study subjects

The present cross-sectional study included persons clinically diagnosed with epilepsy of either gender and more than 18 years of age, receiving conventional and newer AEDs as monotherapy or polytherapy for at least six months, from Neurology Out Patient Department-Epilepsy clinic, All India Institute of Medical Sciences (AIIMS), New Delhi. The AEDs considered were VPA, CBZ, PHT, levetiracetam (LEV), lamotrigine (LTG), and clobazam (CLB). Subjects receiving other than above mentioned AEDs, having dietary/trace element supplements (excluding calcium or folic acid supplement), with signs of malnutrition or dietary restrictions, other neurological diseases/disabilities, other systemic disease, and unwillingness to provide informed consent were excluded from this study. Apparently healthy age and gender matched subjects accompanying the patients and satisfying the eligibility criteria for healthy volunteers were recruited as controls. The participants were enrolled after obtaining written informed consent between December 2011 and November 2012. The study protocol was approved by the Institute Ethics Committee, AIIMS (Reference number: IESC/T-338/02.09.2011). The study was conducted in accordance with Indian Good Clinical Practice guidelines and Declaration of Helsinki.

Detailed medical histories of the subject, geographical and socio-demographic background were recorded and he/she was examined for signs of malnutrition. All the Subjects were in the interictal period and at least 24 h seizures free at the time of blood sampling, and serum was collected according to International Union of Pure and Applied Chemistry (IUPAC) guidelines (Cornelis et al., 1996). Estimation of liver and renal function parameters were performed with an automated chemistry analyzer (Roche Hitachi 912 Chemistry Analyzer, GMI Inc., USA) in the Department of Neuro-Biochemistry, AIIMS, New Delhi.

Trace elements analysis

A complete dissolution of samples was performed using MW 800 Microwave digestion system (Aurora instruments Ltd., Vancouver, Canada). The digestion procedure was optimized as: digestion time (5 min), sample volume (0.5 mL), and oxidant concentration [4 mL suprapure nitric acid (HNO_3) (65%) and 0.5 mL hydrogen peroxide (20%) (Merck Chemicals, India)]. The digested samples were subjected to Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) with filtration.

Aluminium, cadmium, cobalt, copper, iron, magnesium, manganese, nickel, lead, selenium, and zinc were estimated in serum using ICP-AES (Model JY 2000-2, HORIBA JobinYvon, France). Reference ranges of trace elements as reported in literature for western population were also considered for comparison (Alimonti et al., 2005; West Midlands Toxicology Laboratory, 2009) (Table 3).

ICP multi-element standard solution containing 1000 mg/L of 23 elements in 1 mol/L HNO_3 (product no. 1 11355 0100, Batch no. HC061563, Merck Chemicals, Germany) was used as reference standard. Selenium standard (S-9760, Lot no. 15H3423, Sigma Chemicals, USA) was used for selenium estimation separately along with concomitant metal analyzer for improved detection limit. ICP-AES, fitted with a cross flow nebulizer and a quartz spray chamber was used throughout with the conditions of: forward power (1.0 kW); nebulizer flow rate (0.76 L/min); dual detector and sweep/reading of 3, reading/replicate of 3, dwell time (5 s), and integration time (10 s). Wavelength was selected from a predefined set for each trace element using the ICP software version 5.2. The blank solution was run for background correction. For calibration curves, the standard solution mixture was diluted step-wise with 5% HNO_3 in the concentration range of 5—100 PPB. The linearity of the calibration curves was considered to be good (correlation coefficient, $r \geq 0.993$). The limit of detection (LOD), as described in the manufacturer's instrument manual was used for calculations (Table 4). The precision was established by duplicate runs for the same batch of samples.

Statistical analysis

The sample size was estimated to be 21 subjects per group anticipating about 20% variations in serum copper level (mean ± SD, 866.7 ± 200.0 ng/ml) presented in a previous study of persons with epilepsy receiving six months of VPA therapy and considering significance level (alpha) 5% and 80% power (Hamed et al., 2004).

Data was analyzed using Statistical Package for the Social Sciences (SPSS) software version 16.0 (Chicago, IL, USA). The Shapiro—Wilks method was used to check the distribution of data. Study group variables with normal distribution were compared by using analysis of variance (ANOVA) and independent samples *T*-test; for variables without normal distribution Kruskal—Wallis and Mann—Whitney *U* tests were used. Chi square test was used for analysis of categorical variables. Since the metal levels follow a non-normal distribution, median and range were used for comparison. p value < 0.05 was considered statistically significant.

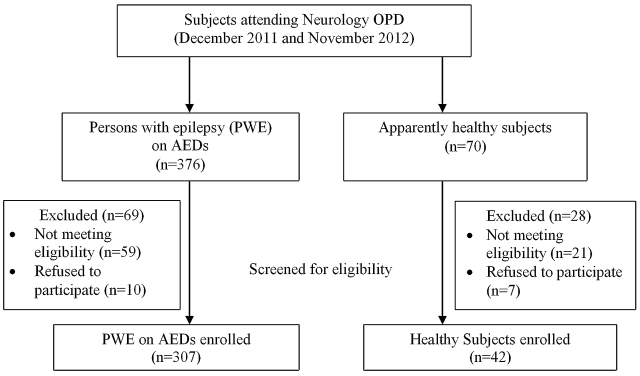

Figure 1 Flow of participants into the study.

Results

Study subjects

On screening of 376 potential subjects with epilepsy, 69 did not meet eligibility criteria, and the rest 307 subjects agreed to participate and were enrolled in the study. A total of 70 age-matched apparently healthy persons were screened and out of these 42 subjects were enrolled as healthy control (Fig. 1).

Out of the total 307 persons with epilepsy, 171 were on monotherapy of AEDs, i.e. VPA ($n = 50$), CBZ ($n = 47$), PHT ($n = 49$), LEV ($n = 21$), and LTG ($n = 4$). None of the subjects were on monotherapy of CLB. According to the pattern of conventional or new AEDs use, the 307 persons with epilepsy were grouped as: Group A: one or more conventional AEDs only (VPA, CBZ, and/or PHT) ($n = 161$); Group B: one or more new AEDs only (LEV, LTG, and/or CLB) ($n = 34$); Group C: polytherapy comprising of both conventional and new AEDs ($n = 112$).

Demographics

There was no significant difference in demographic characteristics between monotherapy groups and control group with respect to age, gender distribution, height, weight, and body mass index (BMI) (Table 1). There was no significant difference in the geographical distribution and economic status among control group and subjects with epilepsy. However, the subjects in the LTG group were having higher mean age, body weight, and BMI, leading to overall significant difference ($p = 0.001$, 0.004 and 0.004, respectively) (Table 1). This could be due to predominance of elderly population and female gender (50%) in the LTG groups. The duration of epilepsy ranged from 0.5 to 40 years and duration of treatment with AEDs ranged from 6 to 180 months. Patient treatment characteristics were presented in Table 2.

The demographic characteristics of groups based on conventional and new AEDs were comparable except that control group was having higher weight (66.3 ± 7.8 kg) and height (166.5 ± 8.9 cm) as compared to other groups ($p = 0.024$ and 0.036, respectively). However, comparison of BMI did not reveal any significant difference among the study groups.

Table 1 Demographic and clinical characteristics of subjects on AED monotherapy and control group.

Patient characteristics	Group 0 (control) ($n=42$)	Group 1 (VPA) ($n=50$)	Group 2 (CBZ) ($n=47$)	Group 3 (PHT) ($n=49$)	Group 4 (LEV) ($n=21$)	Group 5 (LTG) ($n=4$)	p value overall	p value between LEV & individual drug groups	p value between LTG & individual drug groups
% of subjects	19.7	23.5	22.1	23.0	9.8	1.9			
Age (yrs)	29.7 ± 5.4	26.3 ± 10.0	28.4 ± 10.0	26.6 ± 9.5	27.6 ± 9.3	47.2 ± 7.9	0.001	0.002 (vs. LTG)	<0.05 (vs. each group)
Gender: female/male (female%)	22/20 (52.4)	13/37 (26.0)	16/31 (34.0)	16/33 (32.7)	12/9 (57.1)	2/2 (50.0)	NS	NS	NS
Height (cm)	166.5 ± 8.9	164.4 ± 6.6	164.6 ± 6.2	161.6 ± 6.6	161.8 ± 5.3	157.0 ± 9.4	0.004	NS	NS
Weight (kg)	66.3 ± 7.8	61.8 ± 20.5	61.5 ± 12.0	54.9 ± 11.9	57.8 ± 13.9	70.7 ± 7.7	0.004	NS	NS
BMI	23.9 ± 1.9	22.8 ± 6.9	22.7 ± 4.2	20.9 ± 3.9	22.1 ± 5.0	28.7 ± 2.7	0.007	NS	0.023 (vs. PHT)
Epilepsy duration (yrs)	NA	5 (0.5—30)	6 (0.5—26)	2 (0.5—16)	4 (0.5—30)	18 (3—26)	0.040	NS	NS
Type of seizure, number of subjects — n (%)									
GTC	NA	34 (68.0)	14 (29.8)	38 (77.6)	14 (66.7)	0 (0)	<0.0001	NS	NS
Focal	NA	11 (22.0)	33 (70.2)	9 (18.4)	5 (23.8)	4 (100)	<0.0001	NS	NS
Absence	NA	1 (2.0)	0 (0)	1 (2.0)	1 (4.8)	0 (0)	NS	NS	NS
JME	NA	4 (8.0)	0 (0)	1 (2.0)	1 (4.8)	0 (0)	NS	NS	NS

Values are expressed as: mean ± SD, median and range (minimum—maximum), or number of subjects — n (%). BMI, body mass index; GTC, generalized tonic clonic seizure; JME, juvenile myoclonic seizure; VPA, valproic acid; CBZ, carbamazepine; PHT, phenytoin; LEV, levetiracetam; LTG, lamotrigine; CLB, clobazam; NA, not applicable; NS, not significant.

Table 2 Patient treatment characteristics of subjects on AED monotherapy.

Treatment characteristics	Group 1 (VPA) ($n = 50$)	Group 2 (CBZ) ($n = 47$)	Group 3 (PHT) ($n = 49$)	Group 4 (LEV) ($n = 21$)	Group 5 (LTG) ($n = 4$)
Drug treatment	Valproic acid	Carbamazepine	Phenytoin	Levetiracetam	Lamotrigine
Drug dose (mg/day)	800.0 (200—1500)	600 (200—1800)	300 (100—400)	1500 (500—3000)	250 (50—300)
Drug treatment duration (months)	31.5 (6—165)	30 (6—180)	18 (6—144)	12 (6—60)	54 (36—60)
Additional calcium and folic acid supplement, number of subjects, *n* (%)					
Calcium supplement	6 (12.0)	7 (14.9)	5 (10.2)	3 (14.3)	4 (100)
Folic acid supplement	9 (18.0)	5 (10.6)	18 (36.7)	3 (14.3)	4 (100)

Values are expressed as: median and range (minimum—maximum), or number of subjects — *n* (%). VPA, valproic acid; CBZ, carbamazepine; PHT, phenytoin; LEV, levetiracetam; LTG, lamotrigine.

Trace element status in study participants

Table 3 describes the wavelength and LOD for each trace element; LOD for all are <1 ng/ml except for Al and Pb. For the purpose of statistical analysis, values below LOD were treated as zero. Trace elements like Al, Cu, Fe, Se, and Zn could be detected in all subjects, however, Cd, Co, Mn, Ni, and Pb could be detected in some of the subjects only. Cd level was below LOD in 240 subjects out of total 349 subjects (307 persons with epilepsy + 42 healthy control). Similarly, Co level was below LOD in 273 subjects, Mn level was below LOD in 126 subjects, Ni level was below LOD in 334 subjects, and Pb level was below LOD in 207 subjects. In control group Cd could be detected in 1 subject (0.36 ng/ml, i.e. within reference range (<1 ng/ml)), Co and Ni were not detected in any subject; Pb could be detected in 16 subjects only.

Trace element status in study participants of AEDs monotherapy groups

All the monotherapy groups were having Al and Cd concentration higher than control group (for Al, $p < 0.001$) and were above the reference limit also (Fig. 2). The mean serum Co levels were within reference range (0.03—0.41 ng/ml) in all the study groups.

There was no significant difference among the drug treated groups in Cu concentration; however these were significantly higher than control group (Table 4 and Fig. 2). LTG group was having similar Fe level and all other monotherapy groups were having significantly higher Fe concentration than control. There was significant difference between LTG group and VPA group ($p = 0.043$) (Fig. 2).

Mn level was in the reference range (4—12 ng/ml) in control and LEV monotherapy groups; levels in other groups were below reference range. As compared to control group, Mn level was significantly low in CBZ and PHT groups ($p = 0.001$ and 0.003, respectively).

Nickel concentration was detected in two subjects in each of the LEV, CBZ, and PHT groups, and in one subject of VPA group; these were within reference range of 0.13—2.8 ng/ml. There was significantly low Pb concentration in VPA group {0 (0—40.50) ng/ml (median and range)}(mean 5.47 ng/ml) ($p = 0.009$) and PHT group {0 (0—70.61) ng/ml} (mean 4.53 ng/ml) ($p = 0.005$) than control group {0 (0—66.56) ng/ml} (mean 15.85 ng/ml). The mean serum levels of Pb were within reference range (<50 ng/ml) in all the study groups.

Selenium concentration was significantly higher in LEV, LTG, and VPA group than control group and the reference range (70—130 ng/ml). There was no significant difference among AEDs monotherapy groups, except that VPA has higher Se concentration than PHT group ($p = 0.041$) (Table 4 and Fig. 2).

There was no significant difference in Zn levels among AEDs monotherapy groups; however all had significantly higher levels than control group and mean values were within the reference range (700—1600 ng/ml) (Table 4 and Fig. 2).

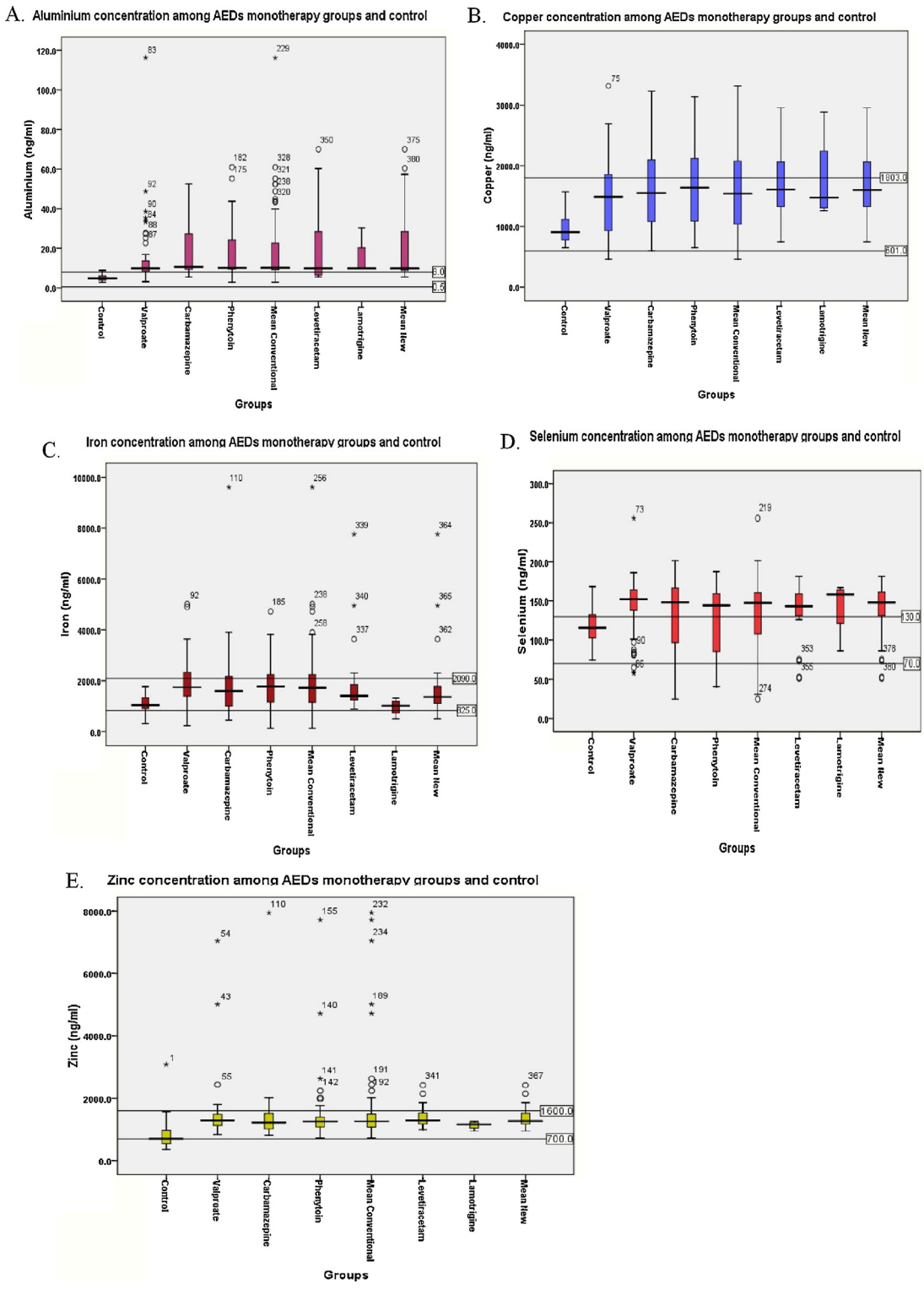

Figure 2 (A) Aluminium, (B) copper, (C) iron, (D) selenium, and (E) zinc concentration among AEDs monotherapy groups and control.

Trace element status in study participants on conventional and new AEDs

The comparison for trace elements level among groups based on conventional and newer AEDs did not reveal any significant difference, except selenium concentration was significantly higher in the combined conventional and new AEDs treated group than only conventional AEDs treated group ($p = 0.010$). All the drug treated groups had significantly higher level of Al ($p < 0.001$), Co ($p = 0.006$), Cu

Table 3 Wavelength, limit of detection and reference range as per previous studies of study trace elements (Alimonti et al., 2005; West Midlands Toxicology Laboratory, 2009).

Element	Wavelength (nm)	Limit of detection (LOD) (ng/ml)	Reference range in healthy adults (ng/ml)
Aluminium	309.271	1.5	0.5–8
Cadmium	228.802	0.35	<1
Cobalt	238.892	0.6	0.03–0.41
Copper	324.754	0.6	601–1803
Iron	259.940	0.5	825–2090
Magnesium	279.553	0.06	17,000–22,000
Manganese	257.610	0.3	4–12
Nickel	221.647	0.7	0.13–2.8
Lead	220.353	5	<50
Selenium	196.026	0.3 (with CMA)	70–130
Zinc	213.856	0.3	700–1600

CMA, concomitant metal analyzer.

($p < 0.001$), Fe ($p < 0.001$), Se ($p < 0.001$), and Zn ($p < 0.001$) than control group. However, Pb concentration was significantly lower in all the drug treated groups as compared to the control group ($p = 0.002$) (Table 5).

Electrolytes level and biochemical parameters in study participants

There was no significant difference among the AEDs monotherapy groups in the concentration of electrolytes, except that LTG group was having significantly lower Ca^{2+} concentration (8.97 ± 0.92 mg/dl) than control group (9.65 ± 0.58 mg/dl) ($p = 0.038$). All the drug treated groups were having lower magnesium level as compared to the control group ($20{,}769.0 \pm 4700.0$ ng/ml) ($p < 0.01$).

The comparison for electrolytes level among groups based on conventional and newer AEDs had shown no significant difference except that magnesium concentration was significantly lower in the combined conventional and new AEDs group than only conventional ($p = 0.014$) and only new AEDs treated group ($p = 0.017$). There was no significant difference between AEDs monotherapy groups and control groups with respect to biochemical tests like liver function tests and renal function tests.

Discussion

The correlation between epilepsy and trace element levels on pathophysiological basis is debatable (Ashrafi et al., 2007; Hunt, 1980; Westbrook and Mayer, 1987), but currently it is of a growing concern as altered element levels has been correlated with epilepsy treatment response (Seven et al., 2013; Wojciak et al., 2013). In a recent study by Seven et al. (2013), it has been concluded that perhaps decreased levels of Zn and Cu could have etiopathophysiologic correlation with drug resistant epilepsy. In another recent study it has been found that children with epilepsy were having increased level of Cu and decreased levels of Fe, Zn, and Cr concentrations (Wojciak et al., 2013). However, there is scarcity of data regarding the effect of newer antiepileptics on any trace element status and the effect of conventional AEDs on trace elements other than Zn, Cu, and Se in persons with epilepsy receiving long term AEDs, which is reflected in the present cross sectional study. In the absence of trace element reference range specific for Indian subjects, the mean serum levels in healthy volunteers were compared as control in this study. However, the reference ranges for healthy adults largely based on western data were also considered for comparison (Alimonti et al., 2005; West Midlands Toxicology Laboratory, 2009). The study identified significant differences in serum trace element levels among study subjects.

In comparison with control group, LEV group had quite higher Zn, Se, Cu, Fe, Al, Cd, Co, and Ni levels. Mn and Pb levels were not significantly different than control group. Other monotherapy groups were having similar metal levels as that of LEV group except that in LTG group Ni and Fe levels were comparable with control group; in VPA group Ni level was comparable and Pb level was significantly lower than control group; in CBZ and PHT groups Ni and Se levels were comparable and Mn level was significantly lower than control. Additionally in PHT group, Pb level was significantly lower than control.

AEDs monotherapy groups' comparison had no significant difference, except that LEV group was having significantly higher Ni level as compared to VPA group; and LTG group had significantly low Fe level as compared to VPA group.

Among the groups based on conventional and new AEDs, there was no significant difference except that combined conventional and new AEDs group had significantly higher Se level than only conventional AEDs group. In comparison with control group, Cd, Mn, and Ni levels have no significant difference, but Pb level was decreased and Zn, Cu, Se, Fe, Al, and Co levels were increased significantly in all these three groups.

Trace elements other than Zn, Cu, and Se are also having significance in the context of epilepsy. Higher aluminium levels in all the AEDs monotherapy groups may menace for Al accumulation and toxicity as it may interact with phosphate containing molecules and enzymes in the body (Baydar et al., 2003). As Cd, Co, and Ni are heavy metals and have been associated with neurological diseases including seizures (Cooper and Legare, 1997; Denays et al., 2005; González-Estecha et al., 2011), so their significant presence can be considered as a signal and may have effect

Table 4 Comparison of copper, selenium, and zinc concentration among AEDs monotherapy groups.

	Groups	Copper					Selenium						Zinc				
		Copper concentration (ng/ml)		Significance (p value) in comparison with			Selenium concentration (ng/ml)		Significance (p value) in comparison with				Zinc concentration (ng/ml)		Significance (p value) in comparison with		
		Mean ± SD	Median (range)	Group 0 (control)	Group 5 (LEV)	Group 6 (LTG)	Mean ± SD	Median (range)	Group 0 (control)	Group 1 (VPA)	Group 5 (LEV)	Group 6 (LTG)	Mean ± SD	Median (range)	Group 0 (control)	Group 5 (LEV)	Group 6 (LTG)
Healthy control	Group 0 (control) (n = 42)	971.5 ± 245.03	908.7 (649.6–1571.1)	–	<0.0001	<0.0001	117.31 ± 21.38	115 .6 (74.6–168.0)	–	<0.0001	0.027	0.041	840.96 ± 470.65	698 .0 (367.8–3084.4)	–	<0.0001	0.011
Conventional drugs	Group 1 (VPA) (n = 50)	1507.0 ± 629.56	1487 (461.5–3315.5)	<0.0001	0.312	0.423	144.22 ± 36.94	151 .9 (57.5–255.6)	<0.0001	–	0.307	0.933	1490.4 ± 1002.55	1293 .7 (836.2–7046.5)	<0.0001	0.804	0.489
	Group 2 (CBZ) (n = 47)	1623.0 ± 664.27	1553 (605.1–3231.2)	<0.0001	0.764	0.667	127.95 ± 50.87	148 .3 (24.5–201.2)	0.210	0.074	0.616	0.579	1413.9 ± 1016.43	1227 .2 (815.4–7945.9)	0.001	0.929	0.593
	Group 3 (PHT) (n = 49)	1662.0 ± 681.37	1641 (650.8–3137.4)	<0.0001	0.940	0.754	127.44 ± 43.47	144 .3 (40.3–187.7)	0.176	0.041	0.532	0.501	1503.9 ± 1090.15	1257 .5 (728.6–7716.6)	<0.0001	0.776	0.509
New drugs	Group 5 (LEV) (n = 21)	1676.0 ± 648.71	1610 (747.2–2954.2)	<0.0001	–	0.787	134.24 ± 37.86	143 .4 (51.3–181.3)	0.027	0.307	–	0.690	1434.2 ± 366.49	1293 .1 (997.7–2419.7)	<0.0001	–	0.129
	Group 6 (LTG) (n = 4)	1775.0 ± 753.68	1478 (1258.5–2884.6)	<0.0001	0.787	–	142.63 ± 37.84	158 .5 (86.2–167.0)	0.041	0.933	0.690	–	1137.9 ± 135.84	1166 .0 (949.9–1269.8)	0.011	0.129	–

VPA, valproic acid; CBZ, carbamazepine; PHT, phenytoin; LEV, levetiracetam; LTG, lamotrigine.

Table 5 Comparison of trace elements concentration among subjects on conventional and new AEDs.

	Group 0 (control) (n = 42)		Group A (conventional AEDs: VPA, CBZ, PHT) (n = 161)		Group B (new AEDs: LEV, LTG, CLB) (n = 34)		Group C (conventional + new) (n = 112)		p value (overall)	p (A vs. B)	p (A vs. C)	p (B vs. C)
Elements level (ng/ml)	Mean ± SD	Median and range	Mean ± SD	Median and range	Mean ± SD	Median and range	Mean ± SD	Median and range				
Al	5.12 ± 1.57	4.8 (2.8–8.8)	16.18 ± 14.40	10.0 (2.8–116.1)	17.59 ± 16.73	10.0 (5.4–70.0)	14.73 ± 17.77	9.7 (1.9–110.8)	<0.0001	0.615	0.477	0.395
Cd	0.01 ± 0.05	0 (0–0.4)	1.46 ± 3.39	0 (0–25.5)	1.12 ± 2.56	0 (0–10.1)	1.27 ± 3.63	0 (0–24.2)	0.074	0.591	0.676	0.788
Co	0.00 ± 0.00	0 (0–0)	0.27 ± 0.53	0.269 (0–3.4)	0.21 ± 0.37	0(0–1.2)	0.19 ± 0.41	0 (0–2.0)	0.006	0.474	0.198	0.956
Cu	971.5 ± 245.03	908.7 (649.6–1571.1)	1602 ± 642.81	1572 (461.5–3315.5)	1687 ± 634.98	1605 (737.4–2954.2)	1576 ± 552.89	1477 (621.1–2914.2)	<0.0001	0.482	0.725	0.363
Fe	1074.0 ± 360.18	1042 (317.6–1765.7)	1955 ± 1263.98	1733 (126.4–9706.7)	1764 ± 1334.01	1386 (506.6–7764)	1804 ± 815.10	1652 (625–4380.4)	<0.0001	0.428	0.231	0.868
Mn	6.22 ± 9.38	4.1(1.0–61.2)	2.44 ± 8.41	0.4 (0–103.4)	5.20 ± 17.13	2.6 (0–101.4)	3.25 ± 14.39	0.66 (0–143.3)	0.232	0.162	0.597	0.548
Ni	0.00 ± 0.00	0(0–0)	0.02 ± 0.12	0 (0–0.8)	0.04 ± 0.17	0 (0–0.8)	0.07 ± 0.26	0(0–1.5)	0.114	0.916	0.477	0.571
Pb	15.8 ± 23.28	0 (0–66.6)	5.70 ± 12.18	0 (0–81.1)	6.16 ± 9.58	0 (0–40.5)	6.24 ± 17.31	0(0–164.5)	0.002	0.892	0.744	0.890
Se	117.31 ± 21.38	115.6 (74.6–168.0)	134.81 ± 43.15	148.3 (24.5–255.6)	135.14 ± 36.90	143.9 (46.4–186.3)	147.90 ± 39.10	153.6 (10.2–219.0)	<0.0001	0.967	0.010	0.087
Zn	840.96 ± 470.65	698.1 (367.8–3084.4)	1486.0 ± 1071.66	1264.9 (728.6–7945.9)	1332.9 ± 328.81	1250.9 (915.6–2419.7)	1537.8 ± 1159.05	1267.3 (762.36–7786.3)	<0.0001	0.411	0.704	0.311

VPA, valproic acid; CBZ, carbamazepine; PHT, phenytoin; LEV, levetiracetam; LTG, lamotrigine; CLB, clobazam; Al, aluminium; Cd, cadmium; Co, cobalt; Cu, copper; Fe, iron; Mn, manganese; Ni, nickel; Pb, lead; Se, selenium; Zn, zinc.

on epilepsy treatment response. Increased Fe level might affect oxidative stress in persons with epilepsy as has been reported with PHT and VPA (Devi et al., 2008). Low Mn level as seen in CBZ and PHT groups has been correlated with convulsions in both humans and animals, though causal relationship is not clear (Gonzalez-Reyes et al., 2007; Critchfield et al., 1993; Dupont and Tanaka, 1985). It has been shown that Pb can decrease thresholds of the electroshock seizure (Cheong et al., 1999) and induce cerebral oedema (Villeda-Hernández et al., 2006). Thus lower lead level in the VPA and PHT group points towards their protective effect.

Higher serum Cu levels obtained in all the AEDs treated groups, is in agreement with previous studies with monotherapy of AEDs (CBZ, phenobarbitone, PHT, and VPA) (Kuzuya et al., 1993; Armutcu et al., 2004; Sözüer et al., 1995; Motta et al., 1998). Enzyme-inducing potential of AEDs leading to increase in hepatic synthesis of ceruloplasmin has been suggested as a possible mechanism (Tutor-Crespo et al., 2003). In contrast, several studies cited reduction in Cu level with CBZ (Hamed et al., 2004) and PHT treatment (Fichsel et al., 1983; Brunia and Buyze, 1972; Tutor et al., 1982) attributing it to enhanced biliary excretion of copper.

Selenium is a part of the GSH-Px enzyme and protects against membrane damage (National Institute of Nutrition, 2009). The unaltered levels of Se among CBZ group is consistent with many studies (Kürekçi et al., 1995; Verrotti et al., 2002), while the significantly higher levels of Se among VPA and newer AEDs monotherapy groups could be attributed to increase in GSH-Px levels (Hamed et al., 2004). However, there are reports of decreased Se levels in VPA-treated animal with clinical manifestations (Hurd et al., 1984).

Increased serum Zn levels in the all AEDs treated groups were in agreement with some previous studies based on VPA treatment (Hamed et al., 2004; Kürekçi et al., 1995), which can be due to increased antioxidant enzymes and Zn being an integral part of these. In contrast, there are reports of reduced Zn level with monotherapy of AEDs (CBZ, PHT, and VPA) (Kuzuya et al., 1993) and with AEDs treatment for $\geq$5 years (Steidl et al., 1987). Though not established, it has been suggested that VPA can bind Zn, replace it from plasma proteins and induce metallothionein synthesis in liver, thus reduce Zn concentration.

Body electrolytes play a vital role for seizure development (Castilla-Guerra et al., 2006; Steidl et al., 1987). However, this study has reported normal levels of electrolytes except reduced serum magnesium levels.

Increase in several trace elements levels in AEDs treated groups might be due to increased absorption of metals from GI tract or decreased elimination from body. Levetiracetam has minimal metabolism and lacks cytochrome P450 isoenzyme-inducing potential (Lyseng-Williamson, 2011). So its pharmacokinetics may have less causative role in affecting the trace elements level than its pharmacodynamic aspects, which needs to be analyzed.

Oxidative stress is a major issue with chronic treatment of AEDs, and heavy metals including Zn, Se are well associated with it (Hamed et al., 2004). Altered metal levels like iron has been linked with intestinal inflammation, intestinal permeability and raised inflammatory markers like IL-6 and C-reactive protein in subjects with epilepsy (Choudhury et al., 2013; Kumar et al., 2013; Alapirtti et al., 2012). These aspects need to be critically investigated with the help of new biomarkers to find out the causal association of altered trace elements level and AEDs treatment.

As the trace elements have a vital role in organ system functioning, the alteration in these associated with AEDs therapy could possibly affect the treatment response and produce multitude of adverse effects, which is to be explored. Limitations of the present study include cross sectional design of the research and lack of previous data and reference ranges specific to Indian population, which precluded accurate sample size determination. Levels of trace elements in serum can vary widely with the dietary habits, industrial exposure, environmental contamination, geographical location, soil, and water treatment etc. Thus, large scale prospective study that compares the pre- and post-treatment levels of the trace elements in persons with epilepsy is required to establish impact of antiepileptics on trace elements in Indian subjects.

Conflicts of interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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