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Elevated serum alkaline phosphatase in epilepsy: effect of age and treatment

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Epilepsy, a common neurological disease characterized by recurrent seizures, affects approximately 69 million people worldwide. Although incurable, seizures may be managed with antiepileptic drugs, the major goal being seizure freedom without adverse side effects. However, the effects of antiepileptic drugs on the haematological and biochemical profiles are subject of debate. Antiepileptic drugs such as phenytoin, carbamazepine, and valproate are the most widely prescribed first-line drugs and are associated with a wide range of adverse effects, such as anaemia, leukocytopenia, eosinophilia, hyperparathyroidism, osteomalacia and hepatobiliary diseases [1,2]. Although newer antiepileptic drugs have less adverse effects, they are generally prescribed in combination with conventional drugs and thus the adverse effects may be due to variable pharmacokinetic interactions [3]. Consequently, these combinations may generate metabolic changes in patients receiving antiepileptic drug therapy, so worsening their quality of life. This emphasizes the need to perform routine monitoring of hematobiochemical indices in clinical practice. Although alterations in these indices have been reported, such studies have been limited by their small sample size and the absence of drug-naïve patients as controls for comparison. Therefore, the aim of this study was to investigate hematobiochemical parameters in patients with epilepsy receiving and not receiving conventional antiepileptic drugs (phenytoin, carbamazepine, and valproate) in either monotherapy or in multitherapy of 2 or 3 drugs.

We conducted a cross-sectional study of patients with epilepsy, all attenders at the Outpatient Department of Neurology, Institute of Human Behaviour and Allied Sciences, Delhi, India. Of the 546 patients, 223 were newly diagnosed and drug-naïve whilst 323 were receiving antiepileptic drug therapy (phenytoin, N = 82; carbamazepine, N = 105; valproate, N = 93; and multitherapy, N = 43). The treatment status of all the patients was confirmed by serum drug quantification as described previously [4]. Patients on drug therapy were receiving the same treatment regimen for at least 2 months to achieve steady-state serum drug levels. A cohort of 37 healthy subjects with no history of any neurological or neuropsychiatric ailment and on no medication including over-the-counter drugs was recruited to provide reference data. All subjects gave written informed consent, and the study was approved by institutional biomedical research ethics committee.

Blood samples were tested for haemoglobin, total leucocyte count, glucose, liver function tests [alkaline phosphatase (ALP), direct bilirubin, total bilirubin, total protein, albumin, globulin, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase] and kidney function tests [uric acid, urea and creatinine] by standard routine methods. Shapiro-Wilk and Levene tests identified a non-normal distribution of the data, therefore, the non-parametric test, Kruskal-Wallis with post-hoc Dunn test. Drug dose and ALP were correlated by Spearman's method. Data are presented as median (interquartile range). $P<0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism 7 and Microsoft Office Excel 2013.

No differences were found in any of the hematobiochemical parameters of patients with epilepsy, except serum ALP levels at 171 U/L vs 80 U/L in the healthy controls ($p<0.0001$), with drug-naïve group comprising the highest percentage (71.3%) of patients with elevated ALP levels (Table 1). Among these drug-naïve patients, individuals having higher pre-treatment seizure frequency had significantly higher ALP levels [pre-treatment seizure frequency: (Daily + Weekly + Monthly) vs (Yearly + 5 yearly), 224 U/L vs 178 U/L, $p = 0.038$]. Furthermore, ALP levels were higher in drug-naïve patients with a more recent seizure episode [last seizure episode: <24 hours vs >24 hours, 228 U/L vs 190 U/L, $p = 0.028$]. ALP levels in patients on valproate monotherapy and on multitherapy were within the normal

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Table 1. Association of demographic and clinical variables with serum ALP levels.

Demographic and Clinical Characteristics	Total	Drug-naïve	Phenytoin	Carbamazepine	Valproate	Multitherapy	P-value	Logistic regression	
								Odds Ratio (95% CI)	P-value
Total patients, *N (%)*	546 (100.0)	223 *(40.9)*	82 *(15.0)*	105 *(19.2)*	93 *(17.0)*	43 *(7.9)*	–	–	–
ALP levels (U/L)	171 (105–261)	201 (142–310)	178 (117–261)	177 (113–255)	92 (67–177)	105 (83–174)	<0.0001	–	–
Normal (40.0–147.0), *N (%)*	230 *(42.1)*	64 *(28.7)*	30 *(36.6)*	42 *(40.0)*	66 *(70.1)*	28 *(65.1)*			
High (>147.0), *N (%)*	316 *(57.9)*	159 *(71.3)*	52 *(63.4)*	63 *(60.0)*	27 *(29.9)*	15 *(34.9)*			
Gender									
Male,	175 (107–255)	198 (142–300)	178 (118–252)	170 (109–243)	94 (69–200)	105 (85–164)	0.860	1.3	0.242
(N)	*(340)*	*(129)*	*(72)*	*(47)*	*(63)*	*(29)*		(0.8–2.1)	
Female,	168 (104–271)	208 (140–327)	187 (80–303)	178 (112–257)	77 (64–130)	107 (69–173)			
(N)	*(206)*	*(94)*	*(10)*	*(58)*	*(30)*	*(14)*			
Age (in years)									
5–15,	338 (199–495)	427 (233–548)	421 (222–464)	304 (183–390)	218 (146–265)	158 (87–175)	<0.0001	8.6	<0.0001
(N)	*(131)*	*(61)*	*(11)*	*(33)*	*(16)*	*(10)*		(4.2–17.7)	
16–19,	169 (115–230)	179 (139–270)	193 (144–260)	166 (124–213)	108 (77–200)	150 (123–154)			
(N)	*(125)*	*(51)*	*(23)*	*(24)*	*(21)*	*(6)*			
20–25,	138 (85–218)	179 (132–231)	168 (112–241)	112 (84–201)	79 (65–126)	88 (65–107)			
(N)	*(144)*	*(50)*	*(28)*	*(20)*	*(36)*	*(10)*			
≥26,	131 (84–194)	162 (122–217)	156 (95–185)	133 (93–172)	68 (62–79)	91 (72–200)			
(N)	*(146)*	*(61)*	*(20)*	*(28)*	*(20)*	*(17)*			
Seizure Type									
Focal/Partial,	181 (114–293)	212 (143–358)	175 (127–259)	170 (107–266)	85 (66–177)	107 (72–175)	0.156	1.2	0.388
(N)	*(211)*	*(79)*	*(33)*	*(61)*	*(10)*	*(18)*		(0.8–1.9)	
Generalized,	165 (103–239)	188 (140–292)	192 (110–252)	172 (110–222)	94 (68–163)	99 (80–167)			
(N)	*(326)*	*(142)*	*(47)*	*(42)*	*(72)*	*(23)*			
Mixed & Others,	229 (115–280)	169 (-)	375 (-)	333 (-)	64 (-)	130 (-)			
(N)	*(9)*	*(2)*	*(2)*	*(2)*	*(1)*	*(2)*			
Epilepsy Type									
Idiopathic,	169 (79–258)	194 (143–250)	204 (120–270)	178 (136–268)	93 (65–167)	93 (74–145)	0.033	1.4	0.243
(N)	*(149)*	*(45)*	*(19)*	*(20)*	*(52)*	*(13)*		(0.8–2.5)	
Symptomatic,	184 (94–284)	192 (109–319)	169 (102–230)	192 (103–255)	104 (83–127)	128 (82–164)			
(N)	*(267)*	*(125)*	*(41)*	*(58)*	*(23)*	*(20)*			
Cryptogenic,	167 (89–241)	210 (109–300)	175 (116–229)	174 (119–244)	105 (74–177)	110 (68–121)			
(N)	*(130)*	*(53)*	*(22)*	*(27)*	*(18)*	*(10)*			
Duration of antiepileptic drug therapy									
2–3 months,	183 (105–283)	-	198 (125–274)	178 (136–304)	94 (68–220)	308 (262–441)	0.035	2.0	0.0017
(N)	*(108)*		*(36)*	*(39)*	*(27)*	*(6)*		(1.3–3.1)	
3–9 months,	136 (85–215)	-	176 (113–285)	195 (129–232)	86 (65–128)	110 (79–166)			
(N)	*(76)*		*(17)*	*(20)*	*(25)*	*(14)*			
9–24 months,	133 (82–236)	-	198 (116–260)	182 (96–254)	85 (66–180)	101 (82–169)			
(N)	*(75)*		*(15)*	*(26)*	*(24)*	*(10)*			
>24 months,	118 (91–170)	-	170 (149–213)	146 (109–212)	90 (68–106)	93 (85–111)			
(N)	*(64)*		*(14)*	*(20)*	*(17)*	*(13)*			

Data are presented in median (interquartile range). P-value is calculated using Kruskal Wallis with post-hoc Dunn test, adjusted by Benjamini-Hochberg FDR and considered significant at $p < 0.05$. The column 'Total' is not included in the statistical analysis. N: number of subjects; PWE: patients with epilepsy; CI: confidence interval.

reference range. Moreover, among the patients on multitherapy, lower ALP levels in patients on multitherapy involving valproate were observed than those on multitherapy excluding valproate (96 U/L vs 172 U/L, $p < 0.0001$). Patients in other groups had high ALP compared to the healthy subjects.

While no gender disparity was observed in the ALP levels, there was a marked effect of age on levels of ALP in all groups, with highest levels in the young (Table 1). Seizure type had no effect on ALP levels, however, significant differences were observed among patients with different epilepsy types. Patients with symptomatic epilepsy were found to have higher levels than those with idiopathic or cryptogenic epilepsies. In addition, patients receiving antiepileptic drug therapy for >3 months had significantly lower ALP levels than those for <3 months (Table 1). There was an inverse correlation between ALP levels and drug doses of phenytoin carbamazepine and valproate (Figure 1). To analyse possible interactions between the parameters, multivariate logistic regression was performed with sex, age, pre-treatment seizure frequency, seizure type, epilepsy type and duration of drug therapy as the independent variables. The analysis retained the effect of age and duration of drug therapy but eliminated the link of epilepsy type with ALP levels (Table 1).

Of all the measured parameters, only serum ALP, a marker of hepatocellular injury and bone turnover, was elevated in patients when compared with healthy subjects. The observation is consistent with previous findings reporting patients with epilepsy to be more susceptible to metabolic bone diseases and fractures linked to elevated serum ALP levels [5,6]. However, the data reported data from patients receiving medication with respect to healthy subjects, instead of drug-naïve patients. This limitation failed to clearly conclude antiepileptic drug administration being the cause of increased ALP levels. To overcome this limitation, we enrolled drug-naïve as well as patients receiving antiepileptic drug therapy. Interestingly, both the groups had increased serum ALP levels, supporting the findings of previous longitudinal studies which reported no change in ALP levels before and after treatment [7,8]. This suggests that it is not the medication but disease phenotype which may play a role in increasing ALP levels. Studies regarding the effect of valproate on bone turnover markers have yielded debatable results in the past. Some studies have reported a marked increase in serum ALP levels by valproate [6,9], but are limited by their small sample size. Consistent with a previous report [10], we note that ALP levels in patients receiving valproate were within the normal reference range suggesting the reduction of epilepsy-linked ALP levels by valproate. This was further strengthened by the observation that patients on multitherapy involving valproate had lower ALP levels compared to patients on multitherapy excluding valproate. Unsurprisingly, we found higher ALP levels in patients aged 5–15 years than in older patients, probably due to the increased osteoblastic activity in children. Though significant association was also observed between epilepsy type and ALP levels with symptomatic epilepsy showing the highest increase, it was not retained after multivariate logistic regression.

Antiepileptic drug therapy has often been associated with osteomalacia due to increased ALP levels [5]. If this had been a relevant mechanism, bone fracture risk in patients with epilepsy should increase with prolonged treatment. However, previous studies have reported contrary outcomes demonstrating prolonged treatment to be associated with lower fracture risk [11,12]. Our study observed higher pre-treatment seizure frequency and recent seizure episodes to be significantly associated with elevated ALP levels, signifying it to be the consequence of seizure occurrence. Additionally, prolonged duration of antiepileptic drug administration decreased the ALP levels in our patient groups and their ALP levels were found to be inversely correlated with the drug doses. Such reports question the role of antiepileptic drugs in altering bone turnover markers. Previously, Hamed et al. [6] investigate several biochemical indices of bone and mineral metabolism such as calcium, magnesium, phosphate, vitamin D, ALP, 25-hydroxy vitamin D (25(OH)D), along with markers of bone remodelling. Hormones such as prolactin, oestrogen, parathyroid hormone, etc. have earlier been reported to influence bone metabolism [10,13] as well as seizure activity [14]. Therefore, future studies should be designed to examine all these parameters along with the bone mineral density to gain insight into the clinical relevance of ALP and associated markers of bone metabolism in epilepsy. We recognize certain limitations. Lacking the bone-mineral density of patients we cannot determine an effect of altered ALP levels on bone mineralization, and as our study is not longitudinal, it lacks follow-up data. However, a strength is the stringent selection criteria, the relatively large overall sample size and the inclusion of drug-naïve patients, although we acknowledge that the sample size in some groups is small.

In conclusion, early age and recent seizure activity were significantly associated with higher ALP levels while prolonged treatment duration and higher doses of antiepileptic drugs were inversely related. Our study suggests increased ALP levels in patients with epilepsy to be an attribute of seizure occurrence and not the consequence of drug administration. This work represents an advance in biomedical science because it demonstrates elevated serum ALP in a large number of drug-naïve patients with epilepsy suggesting higher ALP levels to be attributed to

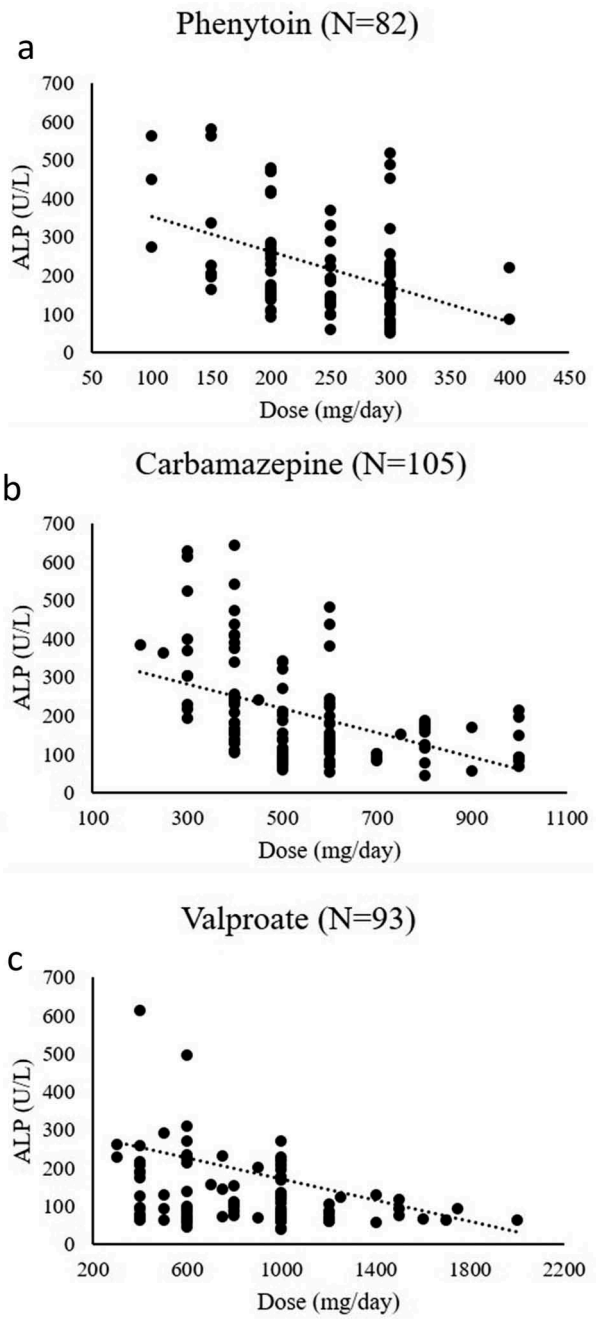

Figure 1. Correlation of serum ALP levels with antiepileptic drug doses: serum ALP levels in patients with epilepsy receiving antiepileptic drug treatment showed significant negative correlation with the doses of all the three antiepileptic drugs, phenytoin ($r = -0.39$, $p<0.001$), carbamazepine ($r = -0.44$, $p<0.001$) and valproate ($r = -0.3$, $p = 0.003$).

seizure occurrence and not the antiepileptic drug therapy.

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Disclosure statement

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