

## **Studies on adverse metabolic effects of antiepileptics and their correlation with blood components.**

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### **Abstract**

**Epilepsy is a neurological disorder knowing no geographical or social boundaries. Majority of patients can be treated with conventional drugs like phenytoin, phenobarbitone and carbamazepine. The present study was planned to correlate the metabolic side effects with the serum drug levels to bridge the existing gap between clinical and biochemical evaluation. Correlation of serum concentration of antiepileptics with thyroid profile, lipid profile, liver enzymes and electrolytes was carried out in 130 known patients of epilepsy on monotherapy or combination therapy. Phenytoin, carbamazepine and phenobarbitone estimation was done using High Pressure Liquid Chromatography. All the groups showed an increase in mean alkaline phosphatase and thyroid-stimulating hormone concentration as compared to healthy age and sex matched individuals. Significant positive correlation was found between serum carbamazepine and serum aspartate transaminase and alanine transaminase concentration ( $p < 0.005$ ). It was concluded that metabolic alterations are mostly mild and clinically insignificant and do not justify routine testing, except in those known to have a coexisting or recently developed hepatic abnormality.**

**Key Words:** Epilepsy, Therapeutic drug monitoring, High Pressure Liquid Chromatography.

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### **Introduction**

Epilepsy is the most common neurological disorder which knows no geographical, racial or social boundaries [1]. Approximately, 70-80% of the patients who develop epilepsy may expect to have their seizures controlled with optimal antiepileptic therapy.

Metabolic side effects of antiepileptic medications have been the cause of debate, whether these drugs require monitoring to assess and interventions to rectify the deranged metabolic markers. Antiepileptics may cause mild increase in liver function tests that tend to resolve over time [2]. Antiepileptic drugs also cause significant reduction in total thyroxine, free thyroxine, free thyroxine index, total triiodothyronine, free triiodothyronine and free triiodothyronine index [3]. These drugs also have effects on lipid profiles [4].

In view of the above-mentioned side effects, therapeutic drug monitoring becomes imperative in treatment of epilepsy. In the present study, we have used Micellar liquid chromatography to separate antiepileptics and tried to correlate the various metabolic side effects with serum levels of phenobarbitone, phenytoin and carbamazepine.

### **Material and Methods**

The present study was carried out at the Department of Biochemistry, Armed Forces Medical College, Pune between June, 2003 to Dec, 2005. One hundred known and twenty three freshly diagnosed patients of epilepsy attending OPD were the subjects for the study. All patients were informed and consent was taken to participate in the study. The present study was cleared by the institutional ethical committee.

5 ml blood samples were collected in the morning during OPD hours in vacutainers. Serum was separated and was analyzed for drug levels using High Pressure Liquid Chromatography (HPLC), estimation of total cholesterol, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) was carried out using Erba- Chem 5, biochemistry analyzer (Transasia Bio Medicals Ltd, Mumbai, India). Analyzer was calibrated every alternate day for the various serum tests according to the manufacturers guideline that included total cholesterol using Cholesterol-DES kit (Source: Erba Manhiem, India), AST & ALT by UV kinetic, Interna-

tional Federation of Clinical Chemistry (IFCC) method using Autopak kit (Source: Bayers Diagnostics; India), ALP by pNPP-AMP (IFCC) kinetic assay using Autospan kit (Source: Bayers Diagnostics; India), IFCC denotes IFCC recommended tests. Thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH) were estimated by a Radioimmunoassay kits (Supplied by Board of Radiation and Isotope Technology, Mumbai).

### Estimations of drug levels

The estimation of Anticonvulsant drugs was done by Micellar HPLC with direct injection of serum samples (5). Samples were diluted with 0.9% saline, filtered and used for HPLC analysis. Carbamazepine and phenytoin were procured from Sigma for the preparation of standards. For standard preparation of Phenobarbital injection Fenobarb (200mg/L) was used. The reagents used to prepare the Micellar mobile phase were Sodium Dodecyl Sulphate (Sigma), 1-butanol (Qualigens), disodium hydrogen phosphate, potassium dihydrogen phosphate and Hydrochloric acid (Merck). Millipore water from Milli Q was used through out the analysis.

The chromatograph (Merck HITACHI) was equipped with a quaternary pump (Lachrom L-7100) and an ultraviolet-visible detector (DAD L- 7455). The flow rate, injection volume, and detection wavelength were 1.0 mL/min, 20 µL, and 220 nm, respectively. Analytical column packed with RP silica gel (Lichrocart C18) was used. The software, HPLC System Manager (Hitachi Chromatography Data Software Station) was used for data handling.

The mobile phase selected as optimum was 0.05 mol/L Sodium Dodecyl Sulphate plus 56.7 mL/L 1-butanol, which gave excellent resolution and allowed the analysis time to be 20 min. All mobile phases contained phosphate buffer at pH 6.88. Calibration curves were constructed for each antiepileptic drug and quantification was also done using internal standard and serum calibration standard procured from Chromsystems.

## Results

Subjects for study were divided into the following groups:

*Group 1:* Patients on monotherapy (phenobarbitone/ carbamazepine/ phenytoin)

*Group 2:* Patients on polytherapy (combination of phenobarbitone and/ or carbamazepine and/ or phenytoin)

*Group 3:* Patients on polytherapy (combination of phenobarbitone and/ or carbamazepine and/ or phenytoin with clobazam and/ or valproate)

60 healthy age and sex matched individuals were included in the study to compare the various metabolic parameters (Table 1).

**Table 1.** Mean values of various metabolic parameters in healthy volunteers.

Metabolic parameters	Mean ± SD
Total Cholesterol	175± 32.8 mg/dl
ALT	21.50± 8.43 IU/L
AST	26.75± 9.19 IU/L
ALP	109.37± 20.0 IU/L
TSH	2.12± 0.929 µg/dl
T3	1.27± 0.42 IU/ml
T4	10.57± 2.61 ng/dl

**Table 2.** Correlation between serum values and various parameters in Group 1

Parameters	Correlation Coefficient	
	Phenytoin	Carbamazepine
Total Cholesterol	0.102	-0.094
AST	-0.078	0.566**
ALT	-0.023	0.693 <sup>#</sup>
ALP	-0.094	0.225
TSH	0.030	0.041
T3	-0.009	-0.162
T4	0.016	0.312

\*\* Significant positive correlation between serum Carbamazepine and serum AST concentration ( $p < 0.005$ ).

<sup>#</sup> Significant positive correlation between serum Carbamazepine and serum ALT concentration ( $p < 0.005$ ).

A higher mean of total cholesterol in group 2 (186.9±53.75 mg/dl) as compared to healthy volunteers (175.85±32.8 mg/dl) was obtained. This could not be attributed to one drug as patients were on polytherapy. While group 1 and 3 had decrease levels as compared to healthy volunteers.

Mean AST levels (28.64 IU/L) and ALT levels (21.96 IU/L) in group 1 were very similar to healthy volunteers but both showed a statistically significant correlation with serum carbamazepine levels ( $p < 0.05$ ). All the groups showed an increase in mean ALP concentration as compared to control (Table 3).

All groups exhibited increase in mean values of TSH compared to healthy volunteers with highest mean in group 3 (3.28±1.74µg/dl). All of the groups showed a statistically insignificant decrease in the mean value of T<sub>4</sub> as compared to healthy volunteers. Group 1 and 2 showed

a decrease in the mean value of T<sub>3</sub> as compared to healthy volunteers while group 3 showed a statistically insignificant increase in mean concentration.

All the groups exhibited an increase in mean ALP concentration as compared to healthy volunteers. Group 1 patient on carbamazepine monotherapy showed positive correlation with serum levels.

**Table 3.** Serum Alkaline Phosphatase in Group 1, 2, 3 and control

Group	No. of patients	ALP (IU/L) Mean ± SD
Control	60	109.37±20.0
Group 1 n = 84	84	187.55±115.38
Group 2 n = 7	7	205.84±115.89
Group 3 n = 32	32	186.86±148.04

## Discussion

The present study showed only a non-significant increase in total cholesterol in those receiving combination therapies while those on monotherapy did not show such increase. There are studies supporting this observation [6-10].

There was an increase in liver enzymes in group 1 which is in agreement with previous studies. This is in conformity with previous studies where serum activities of liver enzymes in patients receiving a long-term anticonvulsant monotherapy were examined retrospectively and it was found that there was a predominant elevation of serum gamma glutamyl transferase (GGT) and ALP and that all enzymes evaluated were more often raised and attained higher values in those receiving phenytoin rather than carbamazepine [11]. Similar enzyme elevations were reported in other studies [12, 13].

There is controversy regarding the exact mechanism for increased enzyme activities. Some studies conclude that increase occurs due to enzyme induction along with liver cell damage [14], while other studies maintain that increase is due to enzyme induction and is mostly mild and clinically insignificant [15]. In our study none of the subjects suffered from liver disease, thus, mild increase found in enzyme levels may only reflect enzyme induction and not hepatocellular damage.

Our observations regarding thyroid profile have also been supported by a number of previous studies. Rousso et al Current Neurobiology Volume 1 Issue 2

(1984) found that phenobarbital receiving patients had low serum T<sub>4</sub> levels and free T<sub>4</sub> indexes but had no changes in TSH concentrations [16]. Similarly, Tiihonen et al (1995) reported that patients receiving anticonvulsant drugs chronically are eumetabolic and do not need thyroxine supplementation [17].

The exact cause for such changes in profile of thyroid function tests is unknown but the probable mechanisms have been studied intensively. Hamada et al (1979) studied effects of diphenylhydantoin on thyroid-hormone binding and concluded that drug induced inhibition of the thyroid hormone binding was probably responsible. While many other studies maintained that increase in conversion and metabolism of the thyroid hormones could explain this effect [18]. The results observed in our study on thyroid profile can thus be explained by interference of drugs with thyroid hormone binding to thyroxine binding globulin and enzyme-induced increased metabolic clearance rate of thyroid hormones.

It could be concluded that metabolic alterations are mostly mild and clinically insignificant and do not justify routine testing, except in those known to have a coexisting hepatic abnormality and those who develop symptoms of hepatic involvement while receiving antiepileptics or develop symptoms of hypothyroidism.

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