These methods could provide successful accuracy, while utilizing common laboratory reagents in a simple, easy to follow procedure. The methods can be successfully applied to routine analysis of formulations containing levofloxacin.


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# Variability of Serum Phenytoin Concentrations in Indian Epileptic Patient Population


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**Due to varied pharmacokinetic behavior and inter individual variation of phenytoin, serum level monitoring is necessary to optimize the drug therapy. The present study was designed to study the relationship between serum levels of phenytoin and different patient related factors like the dose administered, effects of age, sex, control of seizures and the influence of co-medication on the phenytoin concentration. Blood samples were collected from the selected patient group (126 patients) and analyzed using a sensitive HPLC method. Of the 111 patients receiving phenytoin as mono therapy, complete seizure control could be achieved in 80 patients. Seventy four percent of these patients had seizures completely controlled with serum phenytoin level below therapeutic range. The following are the conclusions: In the epileptic patients on monotherapy, the seizure control with phenytoin was observed in the concentration range of 3.9 to 29 μg/ml with a mean (± SD) of 11.56±13 μg/ml. Patients showing therapeutic benefit ranged with serum drug level from sub therapeutic level to toxic level indicating the reference therapeutic range (10 to 20 μg/ml) has to redefined for Indian epileptic patient population.**



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Serum level monitoring of antiepileptic drugs is important for optimizing drug therapy since relationships between serum levels and therapeutic and toxic effects have been clearly established for several drugs. Routine monitoring of

serum levels is especially important since drug bioavailability, as well as the rate of drug metabolism and excretion, varies widely among patients. An increase of the phenytoin dose usually gives an unpredictable increase in the serum levels so that monitoring is necessary to assure that optimal levels are maintained[1]. Phenytoin serum levels within a range of 10–20 μg/ml give satisfactory seizure control in most patients[2-4] and hence it is used as a first line agent in the treatment and prevention of tonic-clonic seizures and other forms of epilepsy[5]. Because of its narrow therapeutic range and saturation kinetics, phenytoin is one of the most suitable drugs for therapeutic drug monitoring (TDM)[6]. Several clinical studies indicate that effective serum level of phenobarbitone and carbamazepine (CBZ) range between 15-40 μg/ml[7] and between 4 and 12 μg/ml[8], respectively.

The present study was designed to study the relationship between serum levels of phenytoin and factors like the dose administered, effects of age, sex, control of seizures and the influence of phenobarbitone and CBZ on phenytoin levels in Indian epileptic patient population.

Phenytoin and phenobarbitone pure samples were gifted by Anglo French Drugs and Industries Ltd., Bangalore and CBZ was procured from INTAS Pharmaceuticals, Ahmedabad. HPLC grade methanol and ethyl acetate were supplied by E. Merck, Mumbai. Glacial acetic acid (HPLC grade) was obtained from S. D. Fine Chem. Ltd., Mumbai.

After due permission from the ethics committee and informed consent of the patients, the following study was undertaken. The patient group consisted of 126 Indian epileptic patients attending the neurology department, government hospital and a multi specialty private hospital in Warangal, AP. Patients were on long term (minimum of two months) oral therapy of phenytoin ('Eptoin', Knoll Pharmaceuticals Ltd.) with a dosage regimen of 3 tablets/day (100 mg morning and 200 (2×100) mg tablets in the night), phenobarbitone ('Gardenal', Rhone-Poulenc Ltd.) with a dosage regimen of two tablets per day (60 mg), and/or carbamazepine ('Tegrital', Novartis) two controlled release tablets per day (400 mg). The information regarding name, age, sex, bodyweight, type of seizures, biochemical and electrophysiological investigations, Electro Encephalogram (EEG), CT Scan report, duration of seizures, first attack, recent attack, frequency of attacks, family history, present treatment with starting date and dose, co medication, side effects, concomitant diseases, date and time of last dose taken and sample time was collected from each patient. One hundred and eleven patients were on phenytoin monotherapy and remaining 15 patients were on polytherapy (7 patients were treated with phenytoin and phenobarbitone and 8 patients were treated with phenytoin and CBZ). Blood samples were collected from each patient prior to morning dose ($C^{SS}_{min}$). Serum samples were stored at −80° until further analysis was done. Samples were measured by a specific and sensitive HPLC method[9]. As serum level/dose ratio expresses the patient's daily serum level concentration per mg/kg of drug administered, it was used as the parameter for comparison[7].

One way analysis of variance in conjunction with Student's t-test for independent samples was performed for statistical analysis of the data and a probability level of $p<0.05$ was chosen as the criterion of statistical significance.

Phenytoin is the most widely prescribed antiepileptic drug in Indian population. Most of the patients had GTCS/CPS/focal seizures/undefined seizures. Of patients treated with phenytoin, 82.8 % patients had no previous history of epilepsy. Fifty percent patients experienced their first attack during childhood itself. Ninety three patients started their treatment after multiple attacks only. With a mean (±SD) dose of 6 (±1.4) mg/kg/day, mean serum level of 13.17 (±11) μg/ml was obtained. Range and mean (±SD) of age, dose, serum level, serum level/dose ratio, body weight and sampling times of patients are given in Table 1. Of total number of patients (126), 27 were children and 99 were adults. Type of seizures and number of patients belonging to each type are shown in Table 2.

TABLE 1: RANGE AND MEAN (±SD) VALUES FOR PATIENTS GIVEN PHENYTOIN

| Parameter | Range | Mean (±SD) |
|---|---|---|
| Age (years) | 1–70 | 26.7 (±13.7) |
| Dose (mg/kg/day) | 2–10 | 6.0 (±1.49) |
| Serum level (μg/ml) | 0.27–50.6 | 13.17 (±11) |
| Serum level/dose ratio | 0.05–6.5 | 2.17 (±1.6) |
| Body weight (kg) | 5–77 | 45 (±11.7) |
| Sampling time (h) | | |

N= number of patients (111)

Though mean serum level/dose ratio increased steadily with age and body weight, the difference was statistically not significant ($p>0.1$). The mean serum level/dose ratio of

TABLE 2: CLASSIFICATION OF THE EPILEPTIC PATIENTS AS PER THE SEIZURE TYPE

| Seizure type | No.of patients | Female | Male | Children | Adult |
|---|---|---|---|---|---|
| | 111 | 41 | 70 | 22 | 89 |
| Generalized tonic clonic seizures | 75 | 30 | 45 | 11 | 64 |
| Complex partial seizures | 11 | 04 | 07 | 05 | 06 |
| Simple partial seizures | 02 | 01 | 01 | 01 | 01 |
| Focal seizures | 09 | 02 | 07 | - | 09 |
| Undefined seizures (epilepsy undetermined whether focal or generalized) | 14 | 04 | 10 | 05 | 09 |

Different types of epileptic seizures were based on the classification of epileptic seizures proposed by the International Classification of the Epilepsies and Epileptic Syndromes (ICEES)

the female and male differ, but the difference was not significant statistically. There was no significant difference between the mean serum level/dose ratios of poor seizure controlled and seizures well controlled groups. Different subgroups (male, female and pediatric, adult groups) also showed no significant difference ($p>0.1$) between mean serum level/dose ratio of poor seizure controlled and seizure well controlled groups.

In case of phenytoin monotherapy, serum levels of phenytoin are necessarily to be within therapeutic range to achieve therapeutic benefit in epileptic patients. Hence all the patients included in the study, were classified according to control of seizures and phenytoin therapeutic range (Table 3). As shown in Table 3, of 111 patients 72.1 % were seizure free and 27.9 % patients had recurrent attacks. Of the 72.1 % subjects (80 patients), the serum drug level ranged from sub therapeutic level to toxic level with a mean (±SD) of 11.56 (±13) μg/ml. As mentioned in literature[10], increased seizure frequency was observed in 27 % patients with drug level beyond therapeutic range (toxic level).

Side effects were observed in 56 patients. Peripheral neuropathy, ataxia, nystagmus, fatigue, drowsiness were common. Diplopia and speech abnormalities were seen in very few (6) patients. Side effects were seen both in patients with drug level within therapeutic range and beyond therapeutic range.

Increased seizure frequency was expected in patients

TABLE 3: CLASSIFICATION OF THE EPILEPTIC PATIENTS ACCORDING TO PHENYTOIN THERAPEUTIC RANGE AND CONTROL OF SEIZURES

| CATEGORY | Within range (10-20 mg/ml) | Beyond therapeutic range Toxic level (>20 mg/ml) | Below therapeutic range Subtherapeutic level (< 10 mg/ml) |
|---|---|---|---|
| TOTAL NO.OF PATIENTS 111 | 38 (34.2 %) | 22 (19.8 %) | 51 (45.9 %) |
| SEIZURE FREE PATIENTS 80 (72.1 %) | 26 (68.4 %) | 16 (72.8 %) | 38 (74.5 %) |
| PATIENTS WITH RECENT ATTACKS 31 (27.9 %) | 12 (31.6 %) | 06 (27.2 %) | 13 (25.5 %) |

Phenytoin (111 patients)

with serum drug levels at sub therapeutic and toxic levels. But therapeutic benefit was observed in patients with serum phenytoin levels below therapeutic range (74.5%) and above therapeutic range (72.8 %). The percentage of patients with therapeutic benefit at lower concentrations (74.5%) was significantly higher compared to patients with therapeutic benefit at concentrations with in therapeutic range (68.4%) suggesting that the patients are seizure free at much lower concentrations and with out any side effect. From our results, lower limit is about 4 μg/ml.

Wrong diagnosis whether sleep disorder or seizures/ inappropriate selection of drug/non-compliance may be the reasons for recurrent attacks in patients with drug levels within therapeutic range and beyond toxic range. Of the remaining individuals with recurrent attacks, 13 had recurrent attacks despite continuing the treatment for more than 3 y. All these patients may be treated with alternate antiepileptic drugs.

Seizures not controlled by monotherapy were treated with polytherapy[11]. Patients suffering from GTCS/CPS/undefined seizures were given polytherapy. Even though more than one anti epileptic drug was taken, seizures weren't controlled in few patients. With approximately same doses being administered to patients with poly therapy as in patients with mono therapy, serum phenytoin levels and phenobarbitone/CBZ levels were reduced. Drug interactions and inter individual variation are perhaps the reasons. Six patients had phenytoin and phenobarbitone at subtherapeutic level. Four patients had one drug (phenytoin/phenobarbitone) at subtherapeutic level and second drug (CBZ) within therapeutic range. Four patients had one drug (phenytoin) at toxic level and other drug (CBZ) within therapeutic range and only one patient had both the drugs (phenytoin and CBZ) with in therapeutic range. This indicates that even though appropriate doses are given to these patients, due to drug interactions drug levels are altered. Hence combination therapy is not of much use in treating epileptic patients. More over it is clear from our study that increasing the serum level beyond 10 μg/ml does not really help in minimizing seizures; on the contrary it may enhance toxicity of the drug. Non-responders may therefore do not benefit from increased dosing of phenytoin nor by the addition of phenobarbitone/ carbamazepine, may however, benefit from a monotherapy with carbamazepine/sodium valproate/a newer drug.

From the above study the following conclusions could be drawn: a) the relationship between the phenytoin dose and concentration is not linear. b) The mean serum level/ dose ratio increased with age but the difference was not statistically significant. c) In Indian epileptic patient population on mono therapy, the effective concentration of phenytoin varied between 3.9 and 29 μg/ml with a mean (±SD) of 11.56±13 μg/ml. The physician may therefore begin the therapy to attain levels in the lower normal range (4-10 μg/ml) since higher doses leading to higher levels do not offer significant seizure control, it is advisable to switch over to another drug rather than increasing the dose. c) Concurrent administration of additional antiepileptic drugs significantly affected the serum level/dose ratio of phenytoin and carbamazepine without offering any clinical advantage.

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