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Therapeutic drug monitoring for antiepileptic drugs using HPLC: An experience at a tertiary care hospital in India

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Abstract

This study was carried out to analyse retrospectively the data of 1,349 patients receiving antiepileptic drugs (AEDs) distributed drug wise into subtherapeutic, therapeutic, toxic and not detectable ranges. Patients were divided into three groups based on the monotherapy they received. In Phenytoin group (n=1255), 26.4% were found to be in therapeutic range, 51.6% in the subtherapeutic range and 20.6% in the toxic range. For Carbamazepine (n=63), 52.4% were in the therapeutic range, 14.3% were in subtherapeutic range, 31.7% in the toxic range and 1.6% were undetectable. Phenobarbitone levels (n=31) were found to be 64.5% in therapeutic range, 22.6% in subtherapeutic range, 9.7% in toxic range and 3.2% in the undetectable range. In 100 patients of phenytoin analyses which were under good seizure control and free of adverse effects, 46% were found to be in therapeutic range, 31% were in subtherapeutic range and 23% were found to be in toxic range. On the basis of this data, it is recommended that therapeutic drug monitoring should be carried out in all patients receiving AEDs for better overall management and long term clinical outcome.

INTRODUCTION

Therapeutic drug monitoring (TDM) is being used to check toxicity, compliance and dose titration in treatment with antiepileptic drugs (AEDs). Since AEDs have a narrow therapeutic index and complex pharmacokinetic properties, wide fluctuations in their plasma concentration can lead to either toxic effects or loss of therapeutic efficacy. The development of technology for quantifying drug concentrations in biological fluids has made it possible to study the relationship between the drug dosage, drug concentration in body fluids and pharmacological effects. It has been seen that the desired therapeutic effect of many AEDs was usually achieved within a specific range of serum concentrations with lower concentrations being more likely to produce an insufficient effect, and higher concentrations being more often associated with adverse effects.[1,2]

Phenytoin (PHT) is used in the treatment of primary or secondary generalized tonic-clonic seizures, partial or complex partial seizures and status epilepticus.[3] It is also the most commonly prescribed AED in our set-up due to its cost effectiveness and easy availability.[4,5] It is not effective for absence seizures. It leads to a reduction in central synaptic transmission, aiding in control of abnormal neuronal excitability.[3] Long term repeated exposure to high serum concentration of phenytoin may predispose patients to irreversible neurotoxicity and may also exacerbate seizures.[6]

Carbamazepine (CBZ) is used for the treatment of generalized tonic- clonic seizures, partial and complex partial seizures. It also modulates the synaptic sodium channels, which prolongs inactivation, reducing the ability of neuron to respond at high frequency.[3] Large interindividual variability in gastrointestinal absorption and metabolism and narrow therapeutic index make this drug a suitable candidate for TDM.[7]

Phenobarbital (PHB) is used for the treatment of all seizures except absence seizures. It reduces synaptic transmission, resulting in decreased excitability of the entire nerve cell inducing sedation. It potentiates synaptic inhibition through action on the γ-aminobutyric acid-A (GABAA) receptor by increasing duration of chloride flow into the synapse.[3]

It has been reported by some authors that there is no significant difference in clinical outcome in

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terms of seizure control and frequency of side effects in patients with doses adjusted on clinical basis alone or by achieving serum levels within predefined target ranges.[8,9] The present study was attempted to retrospectively analyse the TDM data of PHT, CBZ and PHB in patients in whom requisition was made by treating physicians. Levels of PHT were also estimated in a group of patients showing no indications for TDM to get an idea about role of TDM in such patients and to overall, assess the role of TDM of AEDs in our setup.

METHODS

The present study is a retrospective analysis of TDM data of PHT, CBZ and PHB in 1,349 patients of both sexes in age group ranging from one year to 75 years over a period of 4 years (2006-2010). These patients were on follow-up at Pt. B.D.Sharma PGIMS, Rohtak, Haryana. The main indications for carrying out TDM in these patients were uncontrolled seizures, features of toxicity and suspected non-compliance. DPH levels were also estimated in 100 patients of epilepsy receiving PHT alone showing no indication for TDM and were seizure free and adverse effects free for a minimum of two months period. Patients on multiple drugs were excluded from analysis.

The blood samples were collected from patients in the morning just before the next dose was due (trough concentration) except in those patients who had suspected overdose toxicity where immediate sampling was done. The serum was separated immediately and refrigerated (4^0C). Analysis was done weekly in batches but immediately for patients with suspected toxicity using fast elution HPLC (High performance liquid chromatography) by Chromsystems, Germany. Quality control was maintained by running three level control sera provided along with the kits.

The patients were divided into four groups depending on the drug received as monotherapy: Group I (PHT), group II (CBZ) and group III (PHB) and group IV was assigned to patients of PHT estimation without indications. They were further categorised depending upon the plasma concentration of the drugs:

Therapeutic: The plasma levels within normal therapeutic range (10-20 mg/L for PHT, 4-12 mg/L for CBZ and 10-40 mg/L for PHB).[1,3]

Subtherapeutic: The plasma levels below the minimum value of range i.e. below 10 mg/L for PHT, below 4 mg/L for CBZ and below 10 mg/L for PHB.[1,3]

Toxic: The plasma levels more than the maximum value of normal range i.e. more than 20 mg/L for PHT, 12 mg/L for CBZ and more than 40 mg/L for PHB.[1,3]

Not detectable: No levels (<0.001 mg/L) could be detected in the serum.

RESULTS

For PHT levels, out of 308 children 220 were males and 88 were females and out of 947 adults, 605 were males and 342 were females. For CBZ levels, out of 24 children, 14 were males and 10 females and out of 40 adults, 23 were males and 17 were females. For PHB, out of 14 children, 10 were males and 4 were females and out of 17 adults, 13 were males and 4 were females. In group IV patients, out of 50 children, 29 were males and 21 females and out of 50 adults, 34 were males and 16 females. The numbers of patients in therapeutic, subtherapeutic, toxic and not detectable ranges for all the four groups are shown in Tables 1- 4 respectively.

DISCUSSION

Out of 1,349 TDM analyses, 65.6% were from male epileptic patients and 34.4% were female patients. This may be expected because of greater prevalence of epilepsy in male population as compared to females and also because sex ratio is skewed towards males in the population of Haryana.[10] Ninety three percent of total samples were for PHT, 4.7% for CBZ and 2.3% for PHB levels. PHT is the most commonly prescribed drug for treatment of epilepsy in both the adults and paediatric age group. Though it is not the drug of choice but due to low cost and easy availability is being prescribed to majority of patients in our set up.[4,5] PHT levels were found to be in therapeutic range in 26.4% cases. But higher proportion of patients on PHT had levels in the subtherapeutic range (51.6%) and toxic range (20.6%). This could possibly be due to a more complex pharmacokinetic behaviour of PHT in terms of its physicochemical characteristics, saturable kinetics and bioavailability.[11] Various drug interactions may also be the reason for this behaviour.[12,13] PHT binds to plasma protein (mainly albumin) and is subjected to displacement by other drugs which compete for the binding sites on the protein. This fact calls for caution as fall in PHT levels due to drug interaction may be misinterpreted leading to

Table 1: Number of patients showing serum phenytoin levels in therapeutic, subtherapeutic, toxic and not detectable range (n= 1255), Group I

	Subtherapeutic range (<10 mg/L)	Therapeutic range (10-20 mg/L)	Toxic range (>20 mg/L)	Not detectable (<0.001mg/L)
Total 1,255	648 (51.6)	331 (26.4)	259 (20.6)	17 (1.4)
Children (Age 1-14 years) n =308	148 (48.36)	69 (22.55)	89 (29.08)	2 (0.01)
Adults (Age 14-75 years) n = 947	500 (52.8)	262 (27.7)	170 (17.9)	15 (1.6)

Values in parenthesis refer to the percentage of patients

an increase in the prescribed dose which, further, amplifies its toxicity.

CBZ levels were analysed in 63 patients, out of whom 52.4% were in the therapeutic range, 14.3% were in subtherapeutic range, 31.7% were found to be in the toxic range and 1.6% were undetectable. Therapeutic levels were achieved in higher proportion of patients than for PHT. This may be due to more complex pharmacokinetic behaviour of PHT. Large interindividual differences in apparent plasma half life linked to autoinduction and narrow therapeutic range make this drug suitable for monitoring.[14]

PHB levels were analysed in 31 patients, out of whom 64.5% were in therapeutic range, 22.6% were in subtherapeutic range, 9.7% were in toxic range and 3.2% were found to be undetectable. Due to variability in PHB pharmacokinetics, measuring its concentration can be useful for individualizing therapy. Since over time patients develop tolerance to sedative effects of PHB, previously intolerable serum concentrations may become tolerable.[15]

The high percentage of subtherapeutic levels for PHT, CBZ and PHB, may be expected as TDM requisitions were frequently made in patients having uncontrolled seizures. Though presence of uncontrolled seizures do not indicate the levels being subtherapeutic in all cases. Uncontrolled seizures may be due to difficult to treat epilepsy and drug levels may be found within the toxic range as such patients tend to be prescribed increased doses. Poor compliance of patients may also be the reason for the subtherapeutic and undetectable levels in these patients. Poor compliance is a bigger issue in our set up, which mainly caters to rural population, due to poor socioeconomic conditions, illiteracy and dependence on free

Table 2: Number of patients showing serum carbamazepine levels in therapeutic, subtherapeutic, toxic and not detectable range (n= 63), Group II

	Subtherapeutic range (<4 mg/L)	Therapeutic range (4-12 mg/L)	Toxic range (>12 mg/L)	Not detectable (<0.001mg/L)
Total 63	9 (14.3)	33 (52.4)	20 (31.7)	1 (1.6)
Children (Age <14 years) n =23	3 (13.0)	14 (60.9)	6 (26.1)	0
Adults (Age 14-75 years) n = 40	6 (15)	19 (47.5)	14 (35)	1 (2.5)

Values in parenthesis refer to the percentage of patients

Table 3: Number of patients showing serum phenobarbital levels in therapeutic, subtherapeutic, toxic and not detectable range (n= 31), Group III

	Subtherapeutic range (<10 mg/L)	Therapeutic range (10-40 mg/L)	Toxic range (>40 mg/L)	Not detectable (<0.001mg/L)
Total 31	7 (22.6)	20 (64.5)	3 (9.7)	1 (3.2)
Children (Age 1-14 years) n = 14	2 (14.3)	9 (64.3)	3 (21.4)	0
Adults (Age 14-75 years) n = 17	5 (29.4)	11 (64.7)	0	1 (5.9)

Values in parenthesis refer to the percentage of patients

supply of drugs from public hospitals. Assessing compliance on clinical grounds alone can be difficult especially in patients with infrequent seizures or easy to treat epilepsy.

In 100 patients of PHT analyses which were under good seizure control and free of adverse effects, 46% were found to be in therapeutic range, 31% were in subtherapeutic range and 23% were found to be in toxic range. No sample was in the undetectable range because of good compliance of these patients as is expected by the outcome. Though it is difficult to speculate on the reason behind this good response. Whether the dose of AEDs should be maintained within the therapeutic range even if epilepsy is well controlled is still controversial. In a prospective trial, patients with controlled epilepsy but having AED levels in therapeutic range were randomised to receive either the same dose or increased dose to achieve drug levels in the reference range. After a follow-up period of 24 months, no significant difference was observed in both the groups but incidence of toxic effects was found to be more in the group receiving increased dose.[16] It is difficult to conclusively attribute seizure control to PHT at subtherapeutic levels in easy to treat epileptic patients as it has also been reported that spontaneous remission (without treatment) may occur in up to 30% patients.[17,18] Also it has been observed that leaving patients seizure free for two years on low dose AED did not reduce risk for recurrence as compared to no AED.[19] At lower serum levels the half life is also low[3,20] and so levels may fall quickly with missed doses exposing to risk of breakthrough seizures. TDM is also essential to identify the adverse effect threshold of patients before empirically decreasing doses to subtherapeutic levels especially in patients with

Table 4: Number of patients (seizure free and adverse effects free) showing serum phenytoin levels in therapeutic, subtherapeutic, toxic and not detectable range (n= 100), Group IV

	Subtherapeutic range (<10 mg/L)	Therapeutic range (10-20 mg/L)	Toxic range (>20 mg/L)	Not detectable (<0.001mg/L)
Total 100	31 (31)	46 (46)	23 (23)	0
Children (Age 1-14 years) n = 50	19 (38)	21 (42)	10 (20)	0
Adults (Age 15-75 years) n = 50	12 (24)	25 (50)	13 (26)	0

Values in parenthesis refer to the percentage of patients

low baseline frequency of seizures.[1]

Thus, we conclude that TDM appears to be a useful tool in monitoring the doses of AEDs in epilepsy patients but interindividual variation in clinical outcome should be kept in mind while attempting an adjustment of doses.

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