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CORRELATION OF THE SERUM LEVEL OF CARBAMAZEPINE WITH SEIZURE CONTROL AND ADVERSE DRUG REACTIONS AMONG EPILEPTICS IN IBADAN, NIGERIA

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ABSTRACT

Title: *Correlation of serum level of carbamazepine with seizure control and adverse drug reactions among epileptics in Ibadan, Nigeria.*

Background: *Epilepsy is a chronic neurological disorder requiring long-term treatment. Seizure control requires adequate blood levels of anti-seizure drugs. Carbarmazepine is one of the most prescribed antiepileptic drugs in Nigeria. This study was carried out to investigate the correlation between serum levels of carbamazepine and seizure control and adverse drug reactions among epileptics in Ibadan, Nigeria.*

Methods: *In a cross-sectional study, sixty-nine patients with confirmed diagnosis of epilepsy who had been on treatment with carbamazepine alone or in combination with phenytoin for at least one month were enrolled into the study and divided into two groups based on seizure control. Drug level in pre-dose (steady state) venous blood was analyzed using high performance liquid chromatography.*

Result: *The mean serum concentration of carbamazepine (CBZ) and carbamazepine-epoxide (CBZ-EP) was 13.5±9.3µg/mL and 6.34±12.61µg/mL respectively. Patients with good seizure control had mean serum CBZ concentration of 12.7 ± 9.2µg/mL versus 15.02 ± 9.7µg/mL among patients with poor seizure control (P=0.33). The serum concentration of CBZ-EP in patients with good seizure control was 8.05 ± 15.2µg/mL while it was 3.11 ± 3.5µg/mL in the second group (P=0.122).*

Drowsiness was the commonest adverse drug reaction (26.1%) and it did not necessitate withdrawal of the drug.

Conclusion

The study showed that serum level of carbamazepine does not correlate with seizure control and adverse drug reactions.

Key words: *epilepsy, carbamazepine, serum level, seizure control, Nigeria*

INTRODUCTION

Epilepsy is a group of chronic disorders in which the indispensable feature is recurrence of seizures that are typically unprovoked and usually unpredictable [1]. In Nigeria and other developing countries, the condition constitutes a very grave problem and occurs more often than in developed countries [2]. In three community based studies in Nigeria, the prevalence ratios of epilepsy were 5 per thousand in urban population and 6 and 37 per thousand in two rural populations [3, 4]

It is important to classify the kind of seizure in order to choose the most effective therapy. The current

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classification of seizures by the International League against Epilepsy (ILAE) [5, 6] is based on clinical description and electroencephalographic pattern.

Treatment of epilepsy requires long-term administration of antiepileptic drugs [7]. The most commonly used antiepileptic drugs are carbamazepine, phenytoin, phenobarbital and sodium valproate [8, 9]. The ideal antiepileptic drug would suppress all seizures without causing any unwanted effects. Despite adequate treatment with antiepileptic drugs, 20-30% of epileptics do not achieve complete seizure control [10]. Therapeutic drug monitoring (measurement of serum drug concentrations) has greatly improved the management of epilepsy [11, 12].

Carbamazepine, an iminostilbene, is a first line drug used in the management of partial and secondarily generalized seizures. It is also useful in the treatment of generalized seizures, neuropathic pain and manic disorder. The predominant pathway of metabolism involves conversion to the 10,11-epoxide. This metabolite is as active as the parent compound and its concentration in plasma may reach 30-40% of that of carbamazepine especially during concurrent administration of phenytoin and sodium valproate. The therapeutic concentrations are said to be in the range 6 - 14µg/mL. Adverse reactions to carbamazepine are well documented in literature [8, 9, 11], but data on the Nigerian population is lacking.

Studies looking at the correlation of serum level of anticonvulsants and seizure control have found a wide interindividual variation in steady state plasma levels. The serum level of these drugs in majority of cases was found to correlate with seizure control and adverse drug reactions among Omani and Mexican epileptics [13, 14]. The serum level of carbamazepine-10,11-epoxide has been found to correlate with carbamazepine toxicity [15]. It has also been shown that serum carbamazepine epoxide correlates more closely with carbamazepine dose than the serum level of carbamazepine itself [16]. Toxicity has also been demonstrated when serum concentrations of carbamazepine exceed 20µg/mL [9, 15].

There is a dearth of data on serum concentration of carbamazepine among Nigerian epileptics. Efforts in this study were directed at the correlation of the serum levels of carbamazepine with seizure control and adverse drug reactions in Nigerians.

MATERIALS AND METHODS

Study Site

The study was carried out at the medical out patient's clinic of the University College Hospital (UCH), Ibadan, Nigeria State between January 2005 and December 2005. The UCH is a tertiary health facility whose catchment area covers the whole southwestern part of Nigeria. Patients were recruited from those attending the neurology clinic of the medical outpatients departments of the hospital. Ethical approval was provided by the joint University of Ibadan/University College Hospital Ethical Review Committee for the study. The high performance liquid chromatography analysis was done at the Department of Pharmaceutical Chemistry of the Obafemi Awolowo University, Ile-Ife, Nigeria.

Patient enrollment

Patients above 18years of age with clinical features of epilepsy were enrolled into the study. Other inclusion criteria were treatment with carbamazepine for at least one month, availability of informed consent and satisfactory baseline renal and liver functions. Patients were excluded from the study if they were receiving other drugs (not antiepileptics) that could interact and affect the serum level of carbamazepine e.g. cimetidine and erythromycin.

STUDY DESIGN

A cross sectional study was done. Sample size was calculated using Epi-info version 6. 69 patients were allocated into two different groups based on seizure control. Group A was made up of 24 patients with poor

seizure control. Patients included in this group had a frequency of at least a seizure per month in the preceding five months despite regular medication. Group B was made up of 45 patients with good seizure control (had not had seizures in the preceeding 6 months).

CONDUCT OF STUDY

All patients who satisfied the inclusion criteria had detailed clinical evaluation and the findings were entered into a case record form (CRF) specifically designed for the study. The information obtained included the history of the illness and clinical findings on examination. Emphasis was laid on drug history and neurological examination for detection of adverse effects of the antiepileptic drugs. The patients enrolled in this study received carbamazepine (Tegretol^{R}) either in the plain or retard forms and phenytoin (Epanutin^{R}). Five millimetres of venous blood was collected from the patients usually before the morning dose of the medication into non-heparinized tubes. It was assumed that a steady state concentration of the drug would have been achieved in these patients based on the half-life of the drugs and duration of use. Serum was separated by centrifugation and stored at –20^{O}C until assayed.

CHEMICALS AND SOLUTIONS

Carbamazepine (CBZ) was provided by Norvatis Pharma Services (Lagos, Nigeria). Carbamazepine 10,11-epoxide was purchased from Sigma Aldrich (Germany).

Diazepam, used as internal standard (IS) for the control of retention times was purchased from WHO Centre for Chemical Reference Substance, Sweden. The solvents, acetonitrile and methanol were HPLC grade from Sigma Aldrich (Germany). Triethylamine, orthophosphoric acid and sodium hydroxide were all of analytical grade. Distilled water used for preparation of the buffer solution was obtained by means of a Merit W4000 (Bobby Sterlin Ltd, Staffordshire, U.K) water purifying apparatus. Frozen, drug free plasma for calibration curves was obtained from the blood bank of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria.

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

The high performance liquid chromatography apparatus was an Agilent 1100 series (Agilent Technologies Inc, Waldbronn, Germany) consisting of a quaternary pump, manual injector, micro-vacuum degasser and a photodiode-array detector. Carbamazepine and its epoxide metabolite were separated on a Hypersil octadecylsilane(ODS) (C18, 125 x 4.0mm, 5 micron) reversed phased column equipped with a Hypersil BDS (4 x 4mm, 5 micron C18) guard column. The mobile phase was a mixture of phosphoric acid buffer solution with triethylamine, pH adjusted to 3.25 with NaOH and acetonitrile (53:47,v/v). The ultra-violet detector wavelength was 213nm for carbamazepine and carbamazepine-epoxide. This method is a modification of that by Yoshida et al [17].

SAMPLE PREPARATION

Diazepam (20µg/ml) in methanol (100µl) was added to the serum sample (250µl) as internal standard, and then 650µl of acetonitrile was added to precipitate the serum proteins. This mixture was vortex-mixed for 15 seconds and centrifuged at 5000rpm for 15 min. Subsequently, 20µl of the clear supernatant was injected into the HPLC system.

CALIBRATION CURVES

Linearity was tested through analyses of serum calibration standards containing known amounts of six different concentrations of carbamazepine (CBZ) and carbamazepine-epoxide (CBZ-EP). Calibration curves were linear in the concentration range of 0.5-50µg/ml for CBZ and 0.25-10µg/ml for CBZ-EP. The

correlation coefficient (r) for CBZ-EP was 0.980 while that of CBZ was 0.990

EXTRACTION YIELD (RECOVERY)

Absolute recoveries were determined by comparing the peak area of extracted quality control (QC) samples with the peak area of recovery standards at the same nominal concentrations. The overall recovery for CBZ, CBZ-EP and IS from the serum were > 84%.

PRECISION AND ACCURACY

Intra- and inter- day accuracy and precision for CBZ and CBZ-EP was assessed by the QC samples. The coefficients of variation (CV) for intra-day assay for both CBZ and CBZ-EP were between 1.3 and 10.1 %.(acceptable upper limit- 10%). The CV for inter-day assay was generally below this limit except for that at 0.5µg/ml CBZ, which was 16%.

Table 1: Seizure control and gender distribution

Sex	*Good Control*	*Poor Control*	*Total*
Male	21 (46%)	17 (70.8%)	38
Female	24 (54%)	7 (29.2%)	31
Total	45 (100%)	24 (100%)	69

Table 2: Seizure control according to seizure types

Seizure type	*Good control*	*Poor Control*	*Frequency*
Complex partial seizures	16 (66.7%)	8 (33.3%)	24
Complex partial seizures with secondary generalisation	7 (38.9%)	11 (61.1%)	18
Generalised tonic-clonic seizures	21 (80.8%)	5 (19.2%)	26
Simple partial seizures	1 (100%)	0	1
Total	45	24	69

Table 3: Mean serum level of CBZ-EP and CBZ in different groups of patients.

Group of Patients	Mean CBZ conc. (µg/mL)	Mean CBZ-EP conc. (µg/mL)
All 69 patients	13.5±9.3	6.34±12.61
With good seizures control	12.70±9.15	8.05±15.2
With poor seizure control	15.02±9.67	3.11±3.47
Who took CBZ (N = 40) (Mean daily dose- 395mg)	13.46 (±10.2)	3.72 (± 6.5)
Who took CBZ-CR (N= 24) (Mean daily dose -421mg)	13.50 (±8.2)	6.74 (± 11.2)
Who took CBZ/PHT (N= 5) (Mean daily dose -370mg)	13.86 (±9.6)	25.30 (± 32.5)

Normal therapeutic range CBZ: 6-14 µg/mL
Normal therapeutic range CBZ-EP: 0.5-3.0 µg/mL

Table 5: Adverse drug reactions and serum levels of CBZ and CBZ-EP

Adverse drug reactions	*Frequency N (%)*	*Mean CBZ concentration (µg/mL)*	*Mean CBZ-EP concentration (µg/mL)*
Drowsiness	18 (26.1)	11.13	3.72
Learning difficulty	4 (5.8)	9.23	1.25
Cognitive	3 (4.3)	11.53	1.27
Skin rash	1 (1.4)	29.40	2.90

Discussion

The preponderance of males (55.1%) among the patients enrolled in this study is in agreement with previous reports [13, 18] and this has been attributed to higher incidence of head trauma among males. The age range obtained in this study is also in keeping with other reports from developing countries [13, 19]. In developed nations, patients with seizure disorder are usually much older. This is probably due to good pre- and perinatal care, an increase in life expectancy and associated cerebrovascular accidents [20].

Complex partial seizures, with or without secondary generalization (60.9%) was the predominant type. Djibuti and co-workers from Georgia reported a similar trend in their study [21]. Danesi [18] and Ogunniyi *et al* [22] in different studies carried out in Nigeria also found partial seizures to be the most frequent seizure type. These findings could be explained by higher frequencies of birth injury, central nervous system infections and childhood febrile infections. Another possible explanation for this is that most seizures previously classified as primary tonic – clonic generalized are now being recognised as secondarily generalised because of availability of better diagnostic procedures.

However, studies from Oman [13] showed generalized seizures to be slightly more frequent than partial seizures. This is probably due to a high rate of consanguinity among the people of this region. It is of note that more patients with generalized tonic-clonic seizures had better control than those with complex partial seizures. This outcome reflects the general consensus about the prognosis of types of seizures [19, 23], which suggests that prognosis of seizure control, is better in generalized than in partial epilepsy.

Serum concentration of carbamazepine (CBZ) and carbamazepine-epoxide (CBZ-EP)

The mean concentration of CBZ and CBZ-EP obtained in our study are higher than the findings from previous studies [14, 24-26]. It is noteworthy that none of these previous studies were carried out among Nigerians or indeed among Africans. In addition, different chromatographic procedures were employed for these previous studies. Studies conducted in India [27] showed very low serum levels (mean of 2.39±1.37µg/ml) for carbamazepine while the level of it's epoxide metabolite was not measured. This low value is most likely due to the chromatographic procedure adopted which used a detection wavelength of 254nm. It is known from the physicochemical properties of carbamazepine and carbamazepine-epoxide that the most sensitive wavelength for its detection is 213nm hence using a different wavelength will thus reduce the sensitivity.

Relationship between serum levels of carbamazepine (CBZ), carbamazepine-epoxide and seizure control

A wide interindividual variability in the serum level of CBZ and its epoxide metabolite and seizure control was recorded in this study. Findings from this study also suggest that lower CBZ-EP levels are associated with poor seizure control while higher CBZ-EP levels augur well for seizure control.

The importance of protein binding of CBZ and its metabolite in the variability of serum levels cannot be ignored since only the unbound fraction of the drug is considered therapeutically active [28]. However, protein binding evaluation was not carried out in this study.

Another important consideration is that individual genetic differences in the metabolism of CBZ by CYP3A4 and CYP2C8 also contribute to these variations in serum levels of the drug and its metabolite [13]. There is a possibility that some patients with very high levels of CBZ-EP are fast metabolizers of CBZ.

Further studies in pharmacogenomics are needed to explore this finding.

Adverse reaction profile

Drowsiness was the most frequent adverse effect and this is in keeping with results from similar studies [29]. This and other observed adverse effects could also have been due to the disease process *ab initio*. The adverse effects recorded during this study were mild and did not necessitate withdrawal of the drug.

There was no correlation between the serum levels of carbamazepine or carbamazepine-epoxide and the adverse drug reactions. This is consistent with the findings of Pieters and co-workers [30], who also found that there was no correlation between serum level of carbamazepine and its epoxide metabolite and adverse drug reactions

Limitations

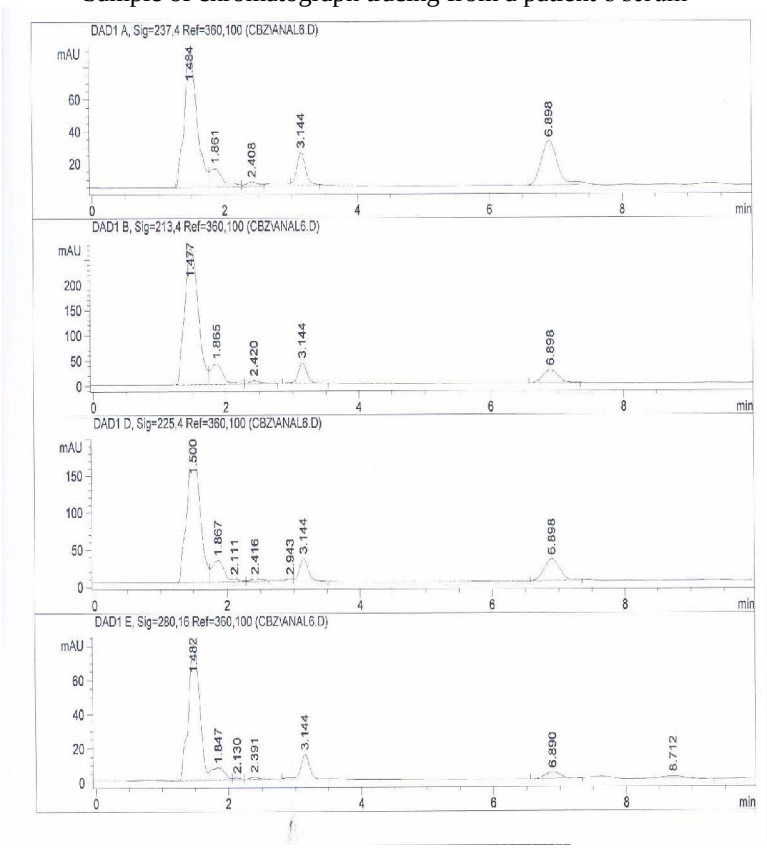
The main limitation of this study was the inability to monitor the compliance of the patients in taking carbamazepine. Pill count was the method used in this study and it has been shown to not totally reliable since it is dependent on the word of the patients. The other limitation was the cost of the chromatographic analysis which was quite high, hence the relatively small sample size. Finally, some of the adverse reactions e.g. drowsiness are mostly subjective feelings reported by the patients hence the possibility of bias.

Conclusion

The serum level of carbamazepine did not correlate with seizure control in patients seen in this study. Since this was an exploratory study in our centre, there is a need for larger multi-centre collaborative studies to investigate the pharmacokinetics of carbamazepine in Nigerian patients with seizure disorders.

Appendix I

Sample of chromatograph tracing from a patient's serum



Sample chromatograph showing some peaks by their retention times. Please note the findings on second block of chromatograph- DAD1B(Sig=213)as specific for the method used.
CBZ – 3.14 minutes; CBZ-EP – 2.42 minutes; DZP (IS) – 6.89 minutes

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