CORRELATION OF SERUM CARBAMAZEPINE AND GAMMA GLUTAMYLE TRANSFERASE IN ADULT EPILEPTICS

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A study was conducted to determine serum carbamazepine (CBZ) levels by high performance liquid chromatography (HPLC) and gamma glutamyl transferase (GGT) activity in 40, already diagnosed epileptic patients (20 males and 20 females) on CBZ therapy for more than six months. Twenty normal healthy controls (JO males and 10 females) were included in the study. The mean f standard deviation of CBZ level in all subjects, males (young and old, females young and old) were 4.2±2.76, 3.47±2.15, 3.65±3.21 and 4.80±^3.27, 3.86 f 3.65 ug/ml respectively.

While the GGT level in all the subjects were 50.57±23.36, in male 52.42 ±23.68 IU/L and in female 48.5±23.28 IIJ/L. It was observed that the serum GT activity> in relation to daily- dosage of CBZ was more in old than young groups. The comparison between daily dosage and CBZ was weakly positive (r-0.2951) while serum CBZ versus serum GGT activity (0.09820), shows no correlation. Hence it was concluded that there is no significant correlation between serum CBZ level and serum GGT activity. To avoid toxic effects of drug over dosage and to obtain therapeutic results drug monitoring is essential.

INTRODUCTION

The history of epilepsy is as long as man himself.[1] Epilepsy affects at least 20-40 million people worldwide. The control of sciezures is essential and most important problem for all patients with epilepsy. CBZ is the drug of choice for the treatment of grandmal epilepsy.[2,3] Its half-life is 15-20 hours in human.[4] The epileptics require long term therapy and therapeutic response of drug decreases due to enzyme induction.[5] In order to get the therapeutic effects the dose is usually increased which again may lead to toxic effects, e.g. agranulocytosis, aplastic anaemia and hepatotoxicity.[6] Drug monitoring is the only remedy to avoid the toxicity of overdosage.[7] There are various methods of drug monitoring like radioimmunoassay (RIA), immunofluorescence, and high performance liquid chromatography.[8] Due to expensive and non-availability of the equipments for drug monitoring at common places, there is need to find a cheap and easily available parameter.[9] Many investigators showed the increase of GGT during anticonvulsant therapy.[10] A significant increase in serum GGT occurs in patients receiving monotherapy for longer periods.[11]

Thus a study was carried to find out whether or not any significant correlation exists between serum CBZ and serum GGT to find out an alternative parameter for serum drug monitoring.

MATERIALS AND METHODS

Forty diagnosed cases of epilepsy (20 males and 20 female) age between 20-65 years taking CBZ monotherapy were selected from different hospitals of Lahore. The patients were divided into:

Group I	Young male 20-29 years
Group II	Old male above 30 years
Group III	Young female 20-29 years
Group IV	Old female above 30 years

4 ml blood was taken aseptically from cubital vein of all the subjects. The serum was separated and stored at -20°C till analysed.

HPLC Apparatus

A Shimadzu HPLC system incorporating an ultraviolet detector (Model SPD 6A Shimadzu Japan) and a reverse phase analytical column (C 18) with computerized data system (Model CR- 4A) were employed.

Reagents

1. Acetonitrile water (450:550 V/V): To 450 ml acetonitrile (HPLC grade Lab Scan Co., Ireland) was

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added 550 ml of water (HPLC grade Lab Scan Co., Ireland) and mix. This was degassed before use.

2. Sodium orthophosphate buffer solution:
17.5 g of disodium hydrogen orthophosphate (Merck. Germany) was dissolved and volume made to 1 litre with HPLC water and then pH was adjusted to 7.0 with 1 MHC1.

3.Internal standard: 20 mg of methyl, phenyl- phenyl hydantoin (Sigma USA) was dissolved in sodium orthophosphate buffer solution in 1 litre volumetric flask and volume was made upto mark.

4. Carbamazepine standard solution: 10 mg of CBZ (Sigma USA) was dissolved in internal standard and volume was made to 1 litre in volumetric flask.

Procedure

Into glass tubes 0.5 ml of serum, 0.5 ml working internal standard and 8 ml diethyl ether were dispensed. A standard tube was prepared using 0.5 ml drug free horse serum, 0.5 ml working standard and 8 ml diethyl ether. All tubes were mixed for 10 minutes on a rotatory mixer and then centrifuged. The ether phase was transferred into glass tubes containing 50_±5 mg of alumina and shaken for 30 seconds. Then solution was transferred into bottle and evaporated to dryness in a water bath at 45°C.

The extract was reconstituted in 100 ul acetonitrile immediately before chromatography and 10 ul was injected onto the column. Chromatography was carried out with a solvent How rate of 3 ml/min and a detector wave length of 250 nm. Drug concentrations were calculated by proportions using peak height ratio with the internal standard.

RESULTS

The serum carbamazepine levels in all patients were 4.24 ± 2.76 ug/ml while in the young male group was 3.47jh2.15 ug/ml and young female group was 4.8CLK3.27 ug/ml. In old male and old female CBZ level was 3.65_± 3.21 and 3.68 ± 3.65 respectively (Table-1).

Table-1: Serum Carbamazepine Level in Patients

Age groups (years)	All subjects mean ± SD (ug/ml)	Male mean ± SD (ug/ml)	Female Mean ± SD (ug/ml)
Young 20-29	4.24 ± 2.76 (1.68-12.70)	3.47 ±2.15 (1.34-9.84)	4.80 ± 3.27 (1.25-12.70)
Young 20-29	3.78 ± 3.32 (0.59-12.24)	3.65±3.21 (0.59-8.21	3.86 ± 3.65 (1.25-12.24)

The serum gamma glutamyl transferase activity in all the patients and controls were 50.57±23.36 Iu/1 and 14.8±6.15 Iu/1 respectively. In male and female patients GGT activity were 52.424±23.86 Iu/1 and 48.5 ± 23.28 Iu/1 respectively (Table-2).

Table-2: Comparison of GGT Activity in Epileptic and Normal Control.

Groups	All subjects mean ± SD (IU/L)	Male mean ± SD (IU/L)	Female mean ± SD (IU/L)
Patients	50.57±23.36 (19-89)	52.42 ± 23.86 (19-96)	48.5±23.28 (11-98)
Control	14.8±6.15 (2.27)	14.2±6.9 (7-27)	15.8 ± 7.29 (2-22
All patients vs all controls	p < 0.001 H.S.	P< 0.001 H.S.	P<0.001 H.S.

Table-3: Percentage of Subjects with Increased GGT Activity in Relation to Daily Dosage of Carbamazepine.

Groups	CBZ (mg/day)	No. of subjects	Parentage
Young	< 600 n = 12	7	58.0
	≥ 600 n = 13	10	76.9
Old	< 600 n = 9	8	88.0
	≥ 600 n = 6	3	50.0

Table-4: Comparison of Daily Dosage and CBZ with GGT in Epileptic Patients.

Group	Dose of CBZ (mg/day)	Serum CBZ level (ug/ml)	GGT (IU/L)	*P* value
Young n = 25	736 ± 206 (400-1000)	4.2 ± 2.76 (1.34-12.70)	52 ± 22.8 (20-96)	-
Old n = 15	493±271 (200-800)	3.78 ± 3.32 (0.59-12.24)	53.5±25.3 (19-98)	-
Dose vs S.CBZ	-	-	-	0.29
S.CBZ vs GGT	-	-	-	0.98
Dose vs GGT	-	-	-	0.20

DISCUSSION

The serum CBZ level in 40 epileptic patients determined by HPLC range 0.59-12.70 ug/ml. The therapeutic range of CBZ is 3.92-9.16 ug/ml. Various investigators have reported the therapeutic ranges of CBZ as Huf and Schain[12] 3 – 15 ug/ml, Donohae[13] 4-10 ug/ml and Mucklow[5] 4- 10 ug/ml respectively.

The GGT level in all the patients ranges 19-98 IU/L the normal values ranges upto 50 IU/L in male and upto 35 IU/L in female. The percentage of patients with raised GGT in relation to daily dosage of CBZ in young and old group was as dose <600 were 7 out of 12 and 8 out of 9, 58% and 88% respectively, while dose >600 mg/day were 10 out of 13 and 3 out of 6 shows 76% and 50% respectively (Table-3). It shows

that the GGT activity increase with the increase of dose specially in adults (30-50 years of age).

The comparison between the daily dosage and serum CBZ level with GGT activity in epileptic shows no significant correlation (Table-4) which confirm the previous studies.[9]

So there is no significant correlation between serum CBZ and GGT activity, while there is weak positive correlation between daily dosage and activity of GGT (Table-4).

It is concluded that to obtain therapeutic results and to avoid the toxicity of over dosage the serum drug level should be done.

REFERENCES

1. Chaudhry MR. History of epilepsy. Lahore: Pakistan Psychiatric Research Centre. 1996.
2. Tomson T. Interdosage fluctuations in plasma carbamazepine concentration determine intermittent side effects. Arch Neurol 1984; 41: 830-34.
3. Miles MV, Lawless ST, Tennison MB. Rapid loading of critically III patients with carbamazepine suspension. Pediatrics 1990: 86:263-66.
4. Shorvan SD. Epilepsy: A general practice perspective. National Society for Epilepsy Research Group London. 1994: 1-58.
5. Mucklow JC. Therapeutic drug monitoring. Medicine International 1992; 5: 4244-48.
6. Marin DB, Greenwald BS. Carbamazepine for aggressive agitation in demented patients during nursing care. Am. J. Psychiatry 1989; 146(6): 805.
7. Bertilsson L, Hojer B. Tybring G, Osterloh J, Rave A. Auto-induction of carbamazepine metabolism in children examined by a stable isotope technique. Clin. Pharmocol. Ther. 1980; 27(l):83-88.
8. Cochran EB, Massey KL, Phelps SJ. A rapid, non-instrumented whole blood carbamazepine assay for pediatric patients. J. Pediatr. 1990; 116(2):307-10.
9. Sheikh SA. Correlation of serum carbamazepine with GGT in children and adolescence by HPLC [Thesis]. Lahore: University of the Punjab, 1996.
10. Aldenhovel HG. The influence of long-term anticonvulsant therapy with diphenyl hydantious and carbamazepine on serum gammaglutamyl transferase, asparate aminotransferase, alanine aminotransferase and alkaline phosphatase Arch Psychiatry Neurol Sci 1988; 237: 312-16.
11. Okesina AB, Donaldson D, Lascelles PT. Serum gammaglutamyl transferase activities in epileptic patients receiving carbamazepine monotherapy. Ann Clin Biochem 1991; 28:207-8.
12. Huf R, Schain RJ. Long-term experiences with carbamazepine (tegretol) in children with seizures. J. Pediatr 1980; 97(2):310-12.
13. Donohoe NV. Epilepsies of childhood. 2nd Ed. London: Butterworth's, 1985:201-13.