CORRELATION OF SERUM CARBAMAZEPINE AND GAMMA GLUTAMYL TRANSFERASE IN ADULT EPILEPTICS

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INTRODUCTION

The history of epilepsy is as long as man himself. Epilepsy affects at least 20-40 million people worldwide. The control of seizures is essential and most important problem for all patients with epilepsy. CBZ is the drug of choice for the treatment of grand mal epilepsy. Its half-life is 15-20 hours in human. The epileptics require long term therapy and therapeutic response of drug decreases due to enzyme induction. 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. Drug monitoring is the only remedy to avoid the toxicity of overdosage. There are various methods of drug monitoring like radioimmunoassay (RIA), immunofluorescence, and high performance liquid chromatography. 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. Many investigators showed the increase of GGT during anticonvulsant therapy. A significant increase in serum GGT occurs in patients receiving monotherapy for longer periods.

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
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 M HC1.

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 up to 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 of 250 nm detector wave length of 250 nm and a flow 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 3.47±2.15 ug/ml and young female group was 4.8±2.15 ug/ml. In old male and old female CBZ level was 3.65±3.21 and 3.68 ± 3.65 respectively (Table-1).

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

The serum gamma glutamyl transferase activity in all the patients and controls were 50.57±23.36 Iu/I and 14.8±6.15 Iu/I respectively. In male and female patients GGT activity were 52.42±23.86 Iu/I and 48.5 ± 23.28 Iu/I respectively (Table-2).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>All subjects mean ± SD (IU/L)</th>
<th>Male mean ± SD (IU/L)</th>
<th>Female mean ± SD (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>50.57±23.36 (19-89)</td>
<td>52.42 ± 23.86 (19-96)</td>
<td>48.5±23.28 (11-98)</td>
</tr>
<tr>
<td>Control</td>
<td>14.8±6.15 (2.27)</td>
<td>14.2±6.9 (7-27)</td>
<td>15.8 ± 7.29 (2-22)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>CBZ (mg/day)</th>
<th>No. of subjects</th>
<th>Parentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young 600</td>
<td>7</td>
<td>58.0</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>10</td>
<td>76.9</td>
<td></td>
</tr>
<tr>
<td>Old 600</td>
<td>8</td>
<td>88.0</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>3</td>
<td>50.0</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of CBZ (mg/day)</th>
<th>Serum CBZ level (ug/ml)</th>
<th>GGT (IU/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young 736 ± 206 (400-1000)</td>
<td>4.2 ± 2.76 (1.34-12.70)</td>
<td>52 ± 22.8 (20-96)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Old 427 (200-800)</td>
<td>3.78 ± 3.32 (0.59-12.24)</td>
<td>53.5±25.3 (19-98)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dose vs S.CBZ</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.29</td>
</tr>
<tr>
<td>S.CBZ vs GGT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.08</td>
</tr>
<tr>
<td>Dose vs GGT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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 Schain12 3 – 15 ug/ml, Donohae13 4-10 ug/ml and Mucklow7 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. 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.

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