Presence of allele CYP3A4*16 does not have any bearing on carbamazepine-induced adverse drug reactions in North Indian people with epilepsy

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Abstract:
OBJECTIVES: The objectives of this study were to determine the relationship between genetic polymorphisms in gene encodings for CYP3A4 and carbamazepine (CBZ)-induced dose-related side effects in North Indian people with epilepsy.

PATIENTS AND METHODS: The current prospective study included 37 patients with CBZ-induced dose-related side effects and 102 patients who did not experience side effects while on CBZ. The genotyping for CYP3A4 allele (CYP3A4*16) was done using real-time polymerase chain reaction (RT-PCR) in Applied Biosystems 7500 RT-PCR System (USA). CBZ was administered in all patients at a dose varying from 15 to 20 mg/kg daily.

RESULTS: Various demographic variables were comparable between the groups except that control of seizures was far better in controls. After testing, it was found that none of our patients had the presence of CYP3A4*16 allele.

CONCLUSION: CYP3A4*16 allele is not represented significantly in North Indian people with CBZ-induced dose-related side effects.

Keywords: Carbamazepine, CYP3A4, epilepsy, dose-related side effects

Introduction
Carbamazepine (CBZ) was first used as an anticonvulsant in the UK in 1965 and in the USA in 1974. It continues to be one of the most commonly prescribed antiepileptic drugs (AEDs) for focal seizures irrespective of the presence or absence of secondary generalization. In addition, it is commonly used in several other diseases including trigeminal neuralgia, other painful neurological conditions, bipolar disorders, and neuromyotonia. Although a highly effective drug for control of seizures at a low cost, its use in clinical practice is restricted by high incidence of adverse effects which can be severe and life threatening in approximately 10%.[1,4]

The side effects related to CBZ can be dose related (type A) which include side effects that are common (1%–10%), predictable, and reversible. These include drowsiness,
lethargy, fatigue, somnolence, ataxia, double vision, tremor, cognitive decline, psychiatric symptoms, and hyponatremia. Alternatively, side effects can be idiosyncratic (type B), which are rare, serious, and life threatening. These include drug rash, Stevens–Johnson syndrome, aplastic anemia, hepatotoxicity, agranulocytosis, and pancreatitis.\(^5\) Whatsoever the mechanism occurrence of any adverse drug reaction (ADR) decreases patient compliance and increases likelihood of discontinuation of therapy and withdrawal seizures. In addition, ADRs contribute significantly to morbidity, increased cost of treatment, and even mortality in people with epilepsy.\(^{[5-7]}\)

Approximately 95% of CBZ is metabolized in the liver to CBZ-10, 11-epoxide (CBZ-E), mainly through activity of cytochrome enzyme CYP3A4. It is also metabolized by cytochrome enzymes CYP2C8 and CYP3A5, albeit to a small extent. Accordingly, genetic variations in activity of CYP3A4 enzyme can affect the clearance of CBZ, resulting in side effects. In fact, one study found the presence of allele CYP3A4*16 to be associated with >50% reduction in metabolism of CBZ in East Asians.\(^{[8]}\) Approximately 1%-5% of Mexican, Korean, and Japanese people have this allele. However, there is no study with regard to this allele in the Indian population. Thus, we planned this study to find the association between CYP3A4*16 allele and CBZ-induced dose-related side effects among people with epilepsy belonging to northern states of India.

**Aims and objectives**

The aim of this study is to determine the relationship between genetic polymorphisms in CYP3A4 gene and dose-related side effects of CBZ.

**Patients and Methods**

The present prospective observational study was conducted at a tertiary care institute in North India from June 2012 to January 2017. During this period, we identified 102 patients with CBZ-induced ADRs. Out of these, 37 were diagnosed to suffer from dose-related ADRs and were analyzed in the final results. All these patients underwent genotyping to determine the type of CYP3A4 allele (CYP3A4*1 wild type or CYP3A4*16). The results were compared with 100 control patients who did not have ADRs with CBZ. Detailed history was obtained from all the patients. All participants were subjected to neurological and systemic examinations. All the details were noted on a predesigned pro forma. All the patients received standard care for ADRs as well for epilepsy. CBZ was administered to all patients at a dose varying from 15 to 20 mg/kg daily. The inclusion criteria included age >18 years and the presence of written informed consent. Exclusion criteria included (a) patients with neurological dysfunction such as subnormal intelligence and weakness, (b) patients with severe liver and kidney dysfunction as well as diabetes mellitus as well as (c) pregnant and lactating women. The Institutional Ethics Committee (histo/14/135/16.1.14) approved the present study. Before recruiting the patients, written informed consent was obtained from each participant.

**Genotyping procedure**

**Blood sampling**

Once enrolled, 4 mL of venous blood was obtained from all the participants. Out of it, 2 mL was placed in ethylenediaminetetraacetic acid (EDTA) vial and kept at −20°C. It was later used for DNA isolation.

**Isolation of DNA from stored blood samples**

Modified phenol-chloroform-isoamyl alcohol method demonstrated by Gill and Werrett was used to separate DNA from venous blood.\(^{[9]}\) To begin with, half milliliter of blood was added to 1 mL of red blood cell (RBC) lysis buffer (1X) followed by vigorous shaking of tubes to lyse RBCs. The tubes were centrifuged at 10,000 rpm for 10 min followed by discarding of supernatant containing lysed RBCs. The pellet thus obtained was again suspended in 1 mL of RBC lysis buffer. The tubes were then centrifuged at 10,000 rpm for 10 min. This procedure was repeated again and again to obtain a clear white pellet of white blood cells. This pellet was then gently mixed with 0.2 M sodium acetate (375 µl), 10% SDS (25 µl), and 20 mg/ml proteinase K (5 µl). This was followed by overnight incubation of mixture at 55°C in a water bath. The lysate thus obtained was then cooled to room temperature and mixed with equal amount of PCI (phenol buffered with Tris-HCl [pH 8.0], chloroform, and isoamyl alcohol in a ratio of 25:24:1). An emulsion was then obtained by gently mixing the contents. This was followed by centrifugation (10000 pm × 5 min) so that solid and liquid phases could be separated. A fresh tube was used to collect liquid phase followed by washing with mixture of chloroform and isoamyl alcohol in the ratio of 24:1. Another fresh tube was then used to obtain supernatant. DNA was then precipitated by adding three volumes of chilled ethanol solution along walls of tubes followed by swirling of tubes. Thereafter, tubes were centrifuged (10,000 rpm × 8 min) followed by decanting of supernatant and air-drying of pellet. Dissolution of air-dried DNA pellet was done in 50 µL of 1X Tris-EDTA buffer of pH 8.0 then that mixture was kept in incubator set at a temperature of 37°C for half an hour. After that, the dissolved DNA was kept at −20°C. NanoDrop method was used to check the quality and quantity of DNA.\(^{[10]}\)

Further genotyping was done using TaqMan probes in Applied Biosystems (ABI) 7500 Real-Time Polymerase Chain Reaction (RT-PCR) System (USA). Each TaqMan
SNP Genotyping Assay (ABI) consists of one tube containing:

a. Two primers which are used to amplify the polymorphic sequence of interest
b. Two TaqMan probes for differentiating between two alleles.

For genotyping, a reaction mixture of 10 µL was constituted. It included 2X Master Mix (5 µL), 20X TaqMan assay (0.5 µL), distilled water (2.5 µL), and DNA (2 µL).

This reaction mixture was added to real-time plates (0.1 µL), which was then kept in RT-PCR plate section. The conditions for RT-PCR were as follows: Preamplification was done at 60°C for 30 s, activation of uracil-N-glycosylase was done at 50°C for 2 min, and DNA polymerase enzyme activation was done at 95°C for 10 min. The denaturation was done at 95°C for 15 s and annealing/extension was performed at 60°C for 90 s. Denaturation and annealing were repeated 45 times.

5’ Nuclease assay

This assay was done to discriminate alleles, i.e., for genotyping. In this assay, results are interpreted through fluorescence of reporter dye which is released from masking effect of quencher dye following cleavage of probes hybridized to complementary sequence. In this assay, specific alleles are detected by observing the fluorescence signal. The relationship between nucleotide sequencing and fluorescence signals is as follows. Fluorescence of VIC dye suggests homozygous allele 1, fluorescence of FAM dye suggests homozygous allele 2, and fluorescence of both VIC and FAM dyes suggests heterozygous alleles 1 and 2 both.

**Statistical analysis**

It was done using SPSS version 24.0 (Statistical Package for the Social Sciences version 24.0) IBM Corp. Released

| Table 1: Comparison of demographic profile among patients with carbamazepine-induced dose-related side effects and patients without carbamazepine-induced adverse drug reactions |
| Parameter | CBZ-induced dose-related side effects (n=37), n (%) | No CBZ-induced side effects (n=102), n (%) | P |
| --- | --- | --- | --- |
| Male sex | 20 (54.1) | 52 (50.1) | NS |
| Mean±SD age (years) | 27.43±9.85 | 27.47±8.74 | NS |
| Mean±SD age of onset of seizure (years) | 17.49±9.77 | 17.51±9.70 | NS |
| Type of seizure | | | |
| Generalized tonic clonic | 12 (32.4) | 30 (29.4) | NS |
| SP | 4 (10.8) | 14 (13.7) | |
| SP with secondary generalization | 11 (29.7) | 32 (31.4) | |
| CP | 5 (13.5) | 11 (10.8) | |
| CP with secondary generalization | 5 (13.5) | 12 (11.8) | |
| Status epilepticus present | 4 (10.8) | 8 (7.8) | NS |
| Family history present | 9 (24.3) | 26 (25.5) | NS |
| Seizure control | | | |
| No recurrence - Excellent | 24 (64.9) | 92 (90.2) | 0.001 |
| 1/6 months - Good | 5 (13.5) | 3 (2.9) | |
| 2/6 months to 1 month - Poor | 4 (10.8) | 1 (0.98) | |
| >1 month - Very poor | 4 (10.8) | 6 (5.9) | |
| Imaging (computed tomography/magnetic resonance imaging) | | | |
| Normal | 14 (37.8) | 42 (41.2) | NS |
| Abnormal | 23 (62.2) | 60 (58.6) | |
| Electroencephalography | | | |
| Normal | 18/26 (69.2) | 45/69 (65.2) | NS |
| Abnormal | 8/26 (30.8) | 24/69 (34.8) | |
| Type of epilepsy | | | |
| Cryptogenic/idiopathic | 14 (37.8) | 41 (40.2) | NS |
| Symptomatic | 23 (62.2) | 61 (59.8) | |
| Side effects | | | |
| Drowsiness | 33 (89.2) | Nil | - |
| Dizziness/vertigo | 11 (29.7) | | |
| Ataxia | 9 (27.3) | | |
| Diplopia/blurring of vision | 8 (21.6) | | |
| Oscillopsia/nystagmus | 1 (2.7) | | |

SP=Simple partial, CP=Complex partial, CBZ=Carbamazepine, NS=Not significant, SD=Standard deviation
Results

Demographic profile
The detailed demographic and clinical profiles of both the groups are given in Table 1. All the demographic and clinical parameters were nonsignificant when compared between cases and controls except for excellent control of seizures which was significantly more in control patients ($P = 0.001$).

Genotype frequency of CYP3A4*1 and CYP3A4*16 in patients on carbamazepine therapy
In the present study, we determined frequencies of different allelic subtypes of CYP3A4. None of the patients in our cohort had CYP3A4*16 allele [Table 2].

Discussion and Conclusion
Ninety-five percent of CBZ is metabolized in the liver through CyP450 system of enzymes, primarily through CYP3A4. However, CYP2C8 and CYP3A5 are also involved to a smaller extent. The main product of CBZ metabolism through the above pathway is a reactive metabolite-CBZ-E. Other metabolites of CBZ metabolism include 2-hydroxy-CBZ and 3-hydroxy-CBZ formed by ring hydroxylation of CBZ. While former is formed though activity of several CYP enzymes, later is formed through activity of CYP2B6 and CYP3A4 enzymes. An arene oxide intermediate is formed during ring hydroxylation. Two-hydroxy-CBZ and 3-hydroxy-CBZ are further metabolized by CYP3A4 into reactive metabolites which can inactivate CYP3A4 and form covalent adducts. Three-hydroxyl-CBZ, and to a minor extent 2-hydroxyl-CBZ, on further metabolism by myeloperoxidase can also produce free radical species which, in turn, results in formation of protein adducts. The formation of protein adducts is supposed to play an important role not only in CBZ-induced hypersensitivity reaction but also for other AEDs. Thus, reduced metabolic activity of CYP3A4 can result in reduced metabolism of CBZ and aggravation of CBZ-induced ADRs.

In the current study, we determined the role of CYP3A4*16 allele in occurrence of CBZ-induced dose-dependent ADRs in the North Indian population. The presence of CYP3A4*16 SNP in Mexican and Japanese populations is associated with decreased catalytic activity of CYP3A4 enzyme. Contrary to previous reports, we did not find the presence of CYP3A4*16 allele in any of our patients despite a reasonably large cohort of patients ($n = 109$). From these observations, it can be concluded that CYP3A4*16 allele is not represented significantly in the North Indian population. A major drawback of the present study is less number of patients. Future studies using bigger sample size will help in better delineation of role of CYP3A4*16 allele in CBZ-induced dose-related adverse effects in Indian people.

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Conflicts of interest
There are no conflicts of interest.

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