Original Article

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Effects of Efavirenz for the Treatment of HIV on the Basis of Genetic Polymorphisms

Internal Medicine Section

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ABSTRACT

Introduction: The CYP2B6 is a liver enzyme that is involved in the metabolism of several drugs including Efavirenz, which is one of the mainstay treatments for Acquired Immunodeficiency Syndrome (AIDS), caused by the Human Immunodeficiency Virus (HIV). Polymorphisms in the CYP2B6 gene determines the rate of enzyme activity. One such polymorphism, rs3745274, where there is a change from G to T at codon 516 results in an amino acid change from glutamine to histidine at position 172. The Food and Drug Administration (FDA) USA has recommended this molecular marker as a companion diagnostic test for Efavirenz.

Aim: To evaluate the effect of Efavirenz in the treatment of HIV on the basis of genetic polymorphisms, to determine the CYP2B6 genotype of healthy individuals and those with HIV before starting the antiretroviral therapy, to review the reported cases of genetic polymorphisms and document drug toxicity in the selected population.

Materials and Methods: A prospective clinical observational study was done at Kamineni Hospital, Hyderabad, Telangana, India, from September 2017 to August 2020 with an inclusion criterion of participants above the age of 18 years who were HIV positive diagnosed by Enzyme Linked Immunosorbent Assay (ELISA) test, reporting to the Outpatient Department (OPD) or getting admitted for treatment with Efavirenz with a sample size of 369 based on the prevalence of CYP2B6 polymorphism in Southern India.

Results: Out of the study population, 276 HIV patients and 93 controls were genotyped by Polymerase Chain Reaction (PCR) and subjected to Restriction Fragment Length Polymorphism (RFLP) technique to establish CYP2B6-516 G>T polymorphism. About 69% were of GT genotype, 18% were of TT genotype and 13% is of GG genotype. Patients with GT genotype were intermediate metabolisers of the drug, those with TT genotype were poor metabolisers of the drug, and GG genotype was an extensive metaboliser of the drug. Individuals with the 516T allele had low enzyme activity and were poor metabolisers of the drug, causing delayed clearance leading to Adverse Drug Reactions (ADR) causing neurological deficits and cardiac complications.

Conclusion: This observation helps the clinician adjust the dose of Efavirenz by studying the genetic polymorphism of the patient. Based on this study, we recommend that all HIV diagnosed patients should undergo CYP2B6 genotyping before starting an Efavirenz based regimen to decrease the adverse drug reactions and promote effective Highly Active Antiretroviral Therapy (HAART) therapy.

Keywords: Acquired immunodeficiency syndrome, Highly active antiretroviral therapy, Human immunodeficiency virus

INTRODUCTION

Human immunodeficiency virus weakens the immune system of the individual and its defence against infections. Helper T cell count usually measures immune function. The stage of advanced disease is Acquired Immunodeficiency Syndrome (AIDS), and it may take a few years to develop if not treated, depending on the individual.

The first few weeks after the infection, people are asymptomatic or have an influenza-like illness which includes fever, headache, rash, or sore throat. As the disease prolongs there is a progressive weakening of the immune system, then they develop other signs and symptoms, like fever, weight loss, lymphadenopathy, cough and diarrhoea.

Efavirenz (EFV), a Non nucleoside Reverse Transcriptase Inhibitor (NNRTI), is commonly used as the first line Antiretroviral Treatment (ART) for HIV globally, particularly in developing countries, owing to its excellent efficacy [1]. World Health Organisation (WHO) recommends Efavirenz in combination with lamivudine and tenofovir as first line antiretroviral treatment [2].

Genetic polymorphism is a distinction in Deoxyribonucleic Acid (DNA) sequence among individuals, races, groups, or ethnicity. Genetic polymorphism of enzymes plays a crucial role in inter individual variations in clinical practice, essential for routine drug prescription [3].

World Health Organisation posited that HIV is a significant public health challenge affecting 33 million lives globally. However, with increased research and effective therapy schedules and plan for HIV treatment and care, it has become a manageable chronic health condition, and enables individuals living with HIV to lead a healthy and longer life span.

Cytochrome P450 (CYP2) B6 is the main part constituent for Efavirenz metabolism, with including accessory pathways involving CYP2A6 and occasionally CYP3A4/5 [4]. Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) are nuclear receptor genes that transcriptionally regulate CYP genes [5]. Efavirenz is glucuronidated by uridine 5′-diphospho-glucuronosyltransferase (UGT) 2B7 directly [6]. Single Nucleotide Polymorphism (SNPs) in the genes that encode these enzymes, especially CYP2B6, will predict higher plasma Efavirenz concentrations [7]. Variations in the enzyme activity due to gene polymorphisms can affect the individual's drug response and can result in adverse drug reactions [8]. The CYP2B6 SNP causing increased plasma Efavirenz concentrations have been already documented, which is related to CNS adverse effects. The present study was conducted with an objective to evaluate the effect of Efavirenz in the treatment of HIV on the basis of genetic polymorphisms. The rationale behind the present study lies in the fact that limited studies till date have been conducted to analyse the association between HIV treatment and genetic polymorphism.

Study objectives:

- To determine the CYP2B6 genotype of healthy individuals in our population and those with HIV before starting the antiretroviral therapy.

- To review reported cases of genetic polymorphisms among the antiretrovirals, especially Efavirenz in HIV patients, and also document drug toxicity of Efavirenz in the selected population.

MATERIALS AND METHODS

A prospective clinical observational study was done at Kamineni Hospital, Hyderabad, Telangana, India from September 2017 to August 2020 with the clearance of Institutional Ethics Committee (Registration No. ECR-58/ Inst/ AP/ 2013/ RR-16) and a sample size of 369, based on the prevalence of CYP2B6 polymorphism in Southern India. Out of the 369-sample size, 276 were HIV positive patients, and 93 were controls. And 207 were males with a mean age of 51 years; 162 were females with a mean age of 45 years.

Inclusion and Exclusion criteria: Participants above the age of 18 years who are HIV positive diagnosed by ELISA test, reporting to the OPD, or getting admitted for treatment on Efavirenz patients who were not willing to participate in the study were excluded.

Study Procedure

All 369 patients enrolled in the study were subjected to PCR-RFLP techinique to establish CYP2B6-516 G>T polymorphism. Out of them 276 were HIV patients and 93 were healthy controls. The study strength were subjected to this technique to maintain the gene haplotype throughout which varied according to the ethnicity. Blood investigations including liver function tests (SGPT,SGOT, cholesterol and triglycerides) and CD4 count tests were done along with assessment of CNS symptoms (hallucinations, insomnia) to find out the association between genotypes and effect of Efavirenz.

Literature Review Methodology

A literature review is defined as academic writing that incorporates current knowledge on a topic, theories, findings, and methodologies [9]. This literature review will provide knowledge on the effect of Efavirenz in the treatment of HIV with the help of genetic polymorphisms based on the primary aim and objective. The objective was to determine how much research has carried out on the effect of Efavirenz on HIV treatment considering genetic polymorphisms.

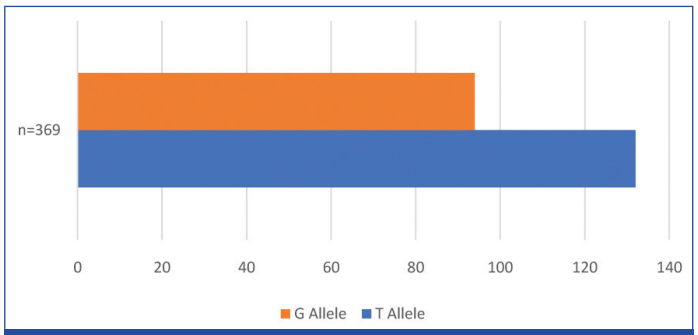
Inclusion and exclusion criteria are used in the elimination and selection of publications [10]. Limiters were applied to the literature review. The articles were limited to 2010-2018 to give current research and differentiate between relevant and irrelevant articles. Screening of the articles was achieved using titles and abstracts relevant to the literature review. Primary research studies were specifically related to HIV and genetic polymorphs. Excluded were the articles without the year of publication and reports not written in the English language since translation would be beyond the scope of the literature review.

RESULTS

Out of the study population, the T-Allele frequency obtained from the sample is 49.07%, and G-Allele was 25.47% [Table/Fig-1]. Allele frequency from the samples does not follow Hardy-Weinberg equilibrium. In the present study population 66 participants out of 369 were TT genotypes who had two copies of T allele each. Thus, in the present study frequency of T allele was 132. Similarly for GG genotype (n=48) the allele frequency was 96.

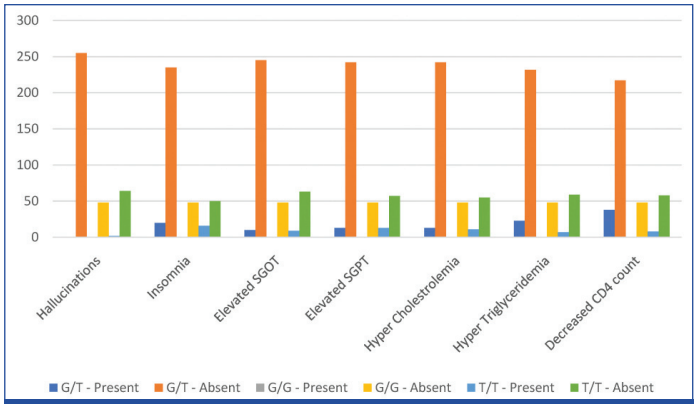
Total 276 HIV patients and 93 controls were genotyped by using PCR and then subjected to Restriction Fragment Length Polymorphism (PCR-RFLP) technique to establish CYP2B6 - 516 G>T polymorphism - 69% GT, 18% TT and 13% GG Polymorphisms. This is explained as follows - 255 were found to be of G/T genotype, 66 of T/T genotype, and 48 of G/G genotype in the study population.

Of the study population of 369, with 66 being T/T genotypes, 2.6% showed hallucinations, and 24% reported insomnia. 19% and 13% of the T/T genotype had deranged Aminotransferases {Glutamic Oxalacetic Transaminase (SGOT) and Glutamic Pyruvic Transaminase (SGPT)}. Elevated cholesterol levels were seen in 16% of the total T/T genotype, along with 11% showing deranged Triglycerides and 12% were showing a decreased change in CD4 cell counts [Table/Fig-1].



[Table/Fig-1]: Allele frequency in study sample- Cases+Control (n=369).

Similarly, within the study population of 369, with 255 being G/T genotypes, no person showed hallucinations, and only 8% reported insomnia. About 4% and 5% of the G/T genotype were having deranged Aminotransferases (SGPT and SGOT). Elevated cholesterol levels were seen in 5% of total G/T genotype, 9% showing deranged Triglycerides, and 15% showing a decreased change in CD4 cell counts [Table/Fig-2].



[Table/Fig-2]: CNS symptoms and deranged lab parameters in patients started on Efavirenz influenced by CYP2B6 polymorphisms (n=369).

But when it came to G/G genotype, which was 13% in the study population (48), there were no reported hallucinations, insomnia, deranged liver enzymes, deranged lipid profile or change in the CD4 cell counts. Deranged triglycerides were seen in 9% of G/T genotype and 11% of T/T genotype, denoting no significant difference among the different genotypes. Also, there is a considerable decrease in CD4 cell count in T/T genotype compared to G/T genotype [Table/Fig-3].

CYP2B6 c.526 G>T	Type of metaboliser	Suggested Efavirenz dose
T/T	Poor	Low-200 mg
G/T	Intermediate	Normal-400 mg
G/G	Extensive	High-600 mg

[Table/Fig-3]: Dose adjustments of Efavirenz based on genotyping.

DISCUSSION

The EFV is associated with several side effects, mainly in the Central Nervous System (CNS). The use of EFV may end in increased neurocognitive effects compared with other antiretroviral drugs [11]. Increased risk of side effects from EFV is associated with genetic polymorphisms in the CYP2B6 gene [12]. Variability in response to EFV is due to the difference in the expression and function of CYP2B6 [12]. Increased plasma concentrations of the drug and the neuropsychiatric effects of EFV are due to CYP2B6 516 G>T polymorphism [12].

Approximately 80% of Efavirenz is metabolised by the hepatic cytochrome P450 enzyme system, of which the phase 1 metabolism isoforms CYP1A2 (8.9%), CYP2C19 (6.8%), CYP2C9 (12.8%),

CYP3A4/CYP3A5 (30.2%), and CYP2D6 (20%) are the most important. Each CYP450 enzyme is encoded by a selected gene, which successively is decided by inherited alleles- one from each parent. Alleles contribute to the observable characteristics known as the individual's phenotype and may either be dominant or recessive [13]. When heterozygous alleles are present, the dominant allele will determine the phenotype.

A study was conducted in Brazil on CYP2B6 516 G>T polymorphism and the CNS side effects in all HIV-positive individuals on Efavirenz treatment [14]. From a study, 225 HIV-positive individuals were prescribed Efavirenz and other medications at a hospital in Rio de Janeiro, Brazil. Eighty-nine cases showed adverse effects of which 43 were with CNS system and 136 controls had none after a minimum treatment of six months. A total of 67 genetic polymorphisms in ABCB1, CYP2A6, CYP2B6, CYP3A4, CYP 3A5, NR1I2 and NR1I3 were selected for the analysis. The analysis showed increased all cause adverse effects related to the CYP2B6 genotype combination 15582CC-516TT-983TT and with the CYP2B6 slow metaboliser group 516TT or 516GT-983CT with an Odd's Ratio equalling to 3.10, and with p-value significance 0.04; CNS adverse effects were nominally related to CYP3A4 rs4646437 with an Odd's Ratio of 4.63, and with p-value significance of 0.014 but without adjusting multiple comparisons.

Another study was done on how genetic polymorphism influences CD4 T cell count in an HIV positive patients who were on antiretroviral therapy in an ethnical region of the Amazon [15]. The CYP2B6 genotyping was performed by RT-PCR (real-time PCR) in 185 patient samples. CYP2B6 G516T allele frequency was 0.36 and varied from the other ethnic groups. The polymorphism seems to have a effect on the response to Efavirenz treatment by reducing CD4+ T cell counts in patients with a high degree of interbreeding of different ethnic genes who used this antiretroviral agent.

Efavirenz crosses the blood brain barrier attaining Cerebrospinal Fluid (CSF) concentrations of 0.5-1.2% corresponding with its plasma concentrations, reaching a therapeutic level in the brain [16]. Research has shown higher rates of neuropsychiatric side effects due to Efavirenz during the first three to four weeks of therapy, including insomnia, vivid dreams, and mood changes and these symptoms lead to discontinuation of the drug by the patients. The plasma levels of Efavirenz over 4 mg/mL have been associated with high toxicity in the central nervous system and when the levels were below 1 mg/mL, it resulted in increase in virological failure of the disease [17].

An increase in half-life of Efavirenz also increases the risk of developing resistance to the drug if it is used as all other ART components. In one study, the polymorphism is associated with CNS toxicities and varying Efavirenz plasma levels, suggesting that prescription of lower dose of the drug in patients presenting T/T genotype could ensure decreased side effects without compromising drug efficacy [18]. However, another study found absolutely no correlation between the plasma levels of the drug and neurotoxicity. Combined drug monitoring for its adverse effects and the genotyping of CYP2B6 has been proposed to decrease viral resistance and toxicity [3].

Genetic polymorphisms of P glycoprotein (P-gp) have been widely studied hence Multidrug Resistance protein 1 (MDR1).3435 C>T and 2677 G>T/A are significantly associated with reduced expression of the protein and several such researches also suggest that these polymorphisms could be related to decreased EFV levels though the results were not conclusive [19]. One study states that though MDR1 variations have been significantly associated with Efavirenz resistance, managing exposure to these levels is not the only pharmacological determinant of resistance, indicating requirement of further studies to show the mechanism by which MDR1 polymorphism work for the virologic response [3].

The ENCORE1 study suggests that the use of a reduced dose universally is non inferior to the standard dose, and the study provides evidence that the dose individualisation based on the genotype can potentially cause a successful viral suppression without unwanted CNS effects which can result in improved outcomes of the patients [20]. Based on the availability of Efavirenz drug in the markets only 200 mg and 600 mg tablets, GG genotype patients can continue their 600 mg daily, whereas TT patients can be asked to take only 200 mg daily. GT patients can take 400 (2×200) mg daily [Table/Fig-3].

However, using these individualised methods through genotyping and supplying reduced dose of Efavirenz while prescribing co-formulations of other nucleoside polymerase inhibitors for a good virological response become a significant inconvenience to the patient and therefore, the implementation of the reduced strength of Efavirenz remains challenging.

Limitation(s)

As a single tertiary care centre study, we cannot extrapolate our results to the general population.

CONCLUSION(S)

The CYP2B6 enzyme is Important for drug metabolism, and its activity shows an inter-individual variability due to polymorphisms. The CYP2B6 G516T polymorphism affects the response to several drugs, including Efavirenz used for the treatment of HIV. Individuals with the 516T allele have low enzyme activity and are poor metabolisers of the drug, causing delayed clearance leading to Adverse Drug Reactions (ADR). From the population analysed for the study, the T allele frequency was higher (0.49) than G (0.25) allele in our study population. HIV patients who have T-allele shows drug-related toxicity more than those who have G-allele. Hence, based on the study, we recommend that patients undergo CYP2B6 genotyping before starting the Efavirenz-based regimen. Larger sample sizes and more trials with the same principle will validate the necessity of using CYP2B6 genotyping as a companion diagnostic test before treating patients with Efavirenz in India.

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