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*Research Article*

# Prevalence of Human Leucocyte Antigen HLA-B*5701 among HIV-1 Infected Individuals in South Eastern Nigeria

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## Abstract

**Background**: Abacavir therapy is one of the Antiretroviral Therapy (ART) used, in conjunction with other antiretroviral agents, to treat people with HIV infection. It is, however, associated with significant risk of hypersensitivity reaction (HSR) among HLA-B*5701-positive patients.

**Aim:** This study was aimed at screening for HLA-B*5701 gene among HIV/AIDS subjects in South-Eastern Nigeria.

**Methods:** A total of 150 subjects (65 males and 85 females) were enrolled in this study. HLA typing was done using the Sequence Specific Oligonucleotide Probes PCR (SSOP-PCR), followed by algarose gel electrophoresis.

**Results:** Results showed that 8 (5.3%) subjects (4 males and 4 females) tested positive for HLA-B*5701 gene. There was no significant association between age, gender, and positivity to HLA-B*5701.

**Conclusion:** We documented a low but medically significant prevalence of HLA-B*5701 in our setting. It was recommended that HLA-B*5701 screening be carried out on HIV/AIDS patients in order to avoid the use of abacavir therapy.

**Keywords**: HLA-B*5701; Abacavir; Hypersensitivity Reaction; Antiretroviral Therapy (ART)

## Introduction

Genetic screening, which is also known as DNA-based testing, is a technique employed in testing for hereditary disorders. It is the analysis of DNA, proteins, and certain metabolites for the purpose of detecting heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical reasons [1]. Genetic screening provide vital information on molecular events that underlie a biological pathway, as well as unraveling gene function [1].

Certain genes code for proteins on the surface of cells. The Human Leucocyte Antigen (HLA) system is the locus of genes that encode for proteins on cell surfaces, primarily responsible for the regulation of immune response in humans [2,3]. HLA alleles are the human version of the major histocompatibility (MHC) complex resident on chromosome 6. There is documented evidence that HLA alleles have been frequently

associated with hypersensitivity reactions to some drug therapy [4].

Adverse drug reactions (ADR) are a major cause of disease and death, worldwide [4].Abacavir therapy is one of the Antiretroviral Therapy (ART) used, in conjunction with other antiretroviral agents, to treat people with HIV infection. It is, however, associated with significant risk of hypersensitivity reaction (HSR) [5]. HIV, a positive single-stranded RNA virus, undergoes reverse transcription to produce double-stranded DNA provirus, which is subsequently incorporated into the host genome and used to produce viral progeny using the host's cellular machinery [6]. Abacavir competes with naturally occurring deoxynucleotides during the viral reverse transcription process. When incorporated into the growing viral DNA strand, abacavir blocks further strand elongation, leading to a premature halt in viral DNA synthesis and chain termination [7].

It is documented that drug-specific T cells, in association with HLA alleles are frequently involved in the pathogenesis of drug HSR. HLA-B*57:01 has the strongest association for abacavir hypersensitivity syndrome and flucloxacillin-induced hepatotoxicity among Western Australian population [4]. Symptoms of abacavir hypersensitivity reaction include skin rash, fever, malaise, gastrointestinal symptoms, and respiratory symptoms. Severe forms of the skin rash may translate into Stevens - Johnson syndrome, toxic epidermal necrolysis, or systemic lupus erythematosus [8]. If a patient experiences a hypersensitivity reaction, Abacavir is discontinued and symptoms generally resolve within 72 hours [9]. Restarting abacavir is contraindicated as it can result in a potentially life-threatening reaction and even death [10].

Despite the fact that HLA-B*57:01 has been documented to be associated with abacavir hypersensitivity reaction in other setting [11], there is no such published data among south-east Nigerians. This is the basis for this research work.

## Materials and Methods

### Subjects and Setting

The cross-sectional observational design was employed in this study; carried out in Enugu, the capital of Enugu State, in the southeastern part of Nigeria. A total of 150 subjects were enrolled in this study. Subjects were all positive to HIV, and were being treated with ART, during the course of the study. Sample size was determined using Gpower statistical software, with a power of 80%.

### Laboratory

### Sample Collection

About 5mls of blood was collected aseptically from the antecubital vein from each of 150 subjects into an EDTA container using standard venepuncture technique [12].

### HLA Typing

Genomic DNA from peripheral blood leucocytes was prepared from Guanidium Isothiocynate (GITC) lysate, by proteinase-K/sodium dodecylsulphate digestion, followed by phenol extraction, ethanol precipitation, and storage in Tris-EDTA, pH 7.4, at 4$^{o}$C [13].

HLA B*5701 typing was amplified by PCR followed by hybridization to sequence-specific oligonucleotide probes (SSOP). Primers used for HLA-B*5701 amplification include B11-AMP and BQ-AMP (Beckman,Palo Alto, CA, USA).Samples (after the PCR process is completed) were then electrophoresed, and DNA fragments visualized by staining with ethidium bromide. HLA B*5701 is positive at base pair 300-350.

### CD4 Count

The CD4 count quantification was performed using Cyflow Counter (Partec, Germany).

### Statistical Analysis

Graphpad Prism 5.0 statistical software was used to analyze the data. Data was group into frequency and mean ± SD for HLA-B*5701 positivity and CD4 count, respectively. Chi-square was used to test for association between each of age, gender, and frequency at 5% probability level.

## Results

**Table 1**. Demographic Details of Subjects.

| Parameter | Frequency (%) | HLA-B*5701 Positivity |
|---|---|---|
| Total | 150 (100.0) | 8 (5.33) |
| Male | 65 (43.3) | 4 (2.67) |
| Female | 85 (56.7) | 4 (2.66) |
| Age Range (yrs) | 18 – 60 | |

**Table 2.** Frequency of HLA B57*01 with Respect to Age and Gender.

| Age Range | Male | | Female | |
|---|---|---|---|---|
| | HLA-B*5701 Positive (%)# | HLA-B*5701 Negative (%) | HLA-B*5701 Positive (%)# | HLA-B*5701 Negative (%) |
| 18-28 | 1(1.5) | 11(16.5) | 0(0.00) | 16(24.0) |
| 29-39 | 2(3.0) | 31(46.0) | 1(1.15) | 36(54.0) |
| 40-50 | 1(1.5) | 11(16.5) | 3(4.5) | 20(30.0) |
| 51-60 | 0(0.00) | 8(12.0) | 0(0.00) | 4(6.0) |

#No significant association observed between Age and HLA B57*01 positivity, $P$ = .31 using Chi-square.

## Discussion

It is well-documented fact that HLA-B*5701 is associated with abacavir hypersensitivity [4]. Much of such literature is focused on Western and American setting, with little or no literature in Nigeria [14]. Thus, this study was aimed at screening for HLA-B*5701 gene among HIV positive subjects in South-Eastern Nigeria.

Results from this study showed that 8 (5.33%) subjects tested positive to HLA-B*5701 Screening, out of a total of 150 subjects studied (Table 1). This is similar to the reported values of 5.33% in Czech Republic by Jilich *et al* [15] and Phillips [16] for US Caucasians (6%–8%) but significantly higher than African Americans (2.5%) as reported by Phillips. In cohorts of African ancestry, HLA B*57 is primarily represented by B*57:03, while B*5702 and B*5701 are present at much lower frequencies; which is consistent with the findings of this study (5.33%, Table 1) [17].

There was, however, no significant association between age and positivity to HLA-B*5701 testing ($P$ = .31, Table 2). This agrees with the fact that the HLA genes are inherited as a single unit in Mendelian fashion, and may not be altered, under normal conditions, with advances in age [18]. HLA-B*5701 screening is supposed to be useful even in countries with a low prevalence of HLA-B*5701, because it may reduce clinical over diagnosis of Abacavir hypersensitivity reactions. HLA-B*5701 screening did not significantly decrease the prevalence of clinical diagnosis of abacavir hypersensitivity, but a negative HLA-B*5701 test result cannot absolutely rule out abacavir hypersensitivity [19] because an immunologically confirmed case of abacavir hypersensitivity in patients without HLA-B*5701 has been reported by Waters *and co-workers* [20].

## Conclusion

Our study had documented a low but medically significant prevalence of HLA-B*5701 in our setting. It is therefore recommended that HLA-B*5701 screening be carried out on HIV/AIDS patients in order to avoid the use of abacavir therapy. This would minimize both cost of treatment and ultimately life threatening adverse drug reactions that may occur following adverse drug reaction.

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