Original Article

ISSN: 1735-0344 *Tanaffos 2016; 15(1): 48-52*

TANAFFOS

Prevalence of HLA-B*5701 and Its Relationship with Abacavir Hypersensitivity Reaction in Iranian HIV- Infected Patients

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Received: 26 September 2015
Accepted: 16 December 2015

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Background: Hypersensitivity reaction (HSR) is a major adverse effect of abacavir (ABC), which occurs in 5-8% of Caucasians. The relationship between Human Leukocyte Antigen (HLA) and ABC HSR has been reported in various populations. It has been proposed to administer ABC only to HLA-B*5701 negative patients to avoid this reaction.

The purpose of this study was to assess the prevalence of HLA-B*5701 in Iranian HIV positive patients. We also sought to find the relationship between this allele with ABC HSR in patients who received the medication.

Materials and Methods: We screened patients for HLA-B*5701 allele using SybrGreen real time PCR-melting method on blood samples from HIV positive patients who were referred to our hospital. The quality of the extracted genome was evaluated by B-globin housekeeping gene as internal control prior to HLA-B*5701 allele screening.

Results: Of 198 HIV-infected patients, 6 (3.0%) had the HLA-B*5701 allele (95% CI, 1%–5%). Among the 28 patients who were given ABC, one individual had the HLA-B*5701 allele and experienced ABC HSR.

Conclusion: Prevalence of HLA-B*5701 in Iranian patients was lower than that in Caucasians but was comparable with that of other Middle Eastern populations. Screening for HLA-B*5701 before ABC administration as part of antiretroviral therapy may reduce the risk of HSR.

Key words: Abacavir, Antiretroviral therapy, HIV,HLA-B*5701 allele, Hypersensitivity reaction, Iran

INTRODUCTION

Abacavir (ABC) is a nucleoside analogue reverse transcriptase inhibitor (NRTI) with activity against human immunodeficiency virus (HIV) (1). It is administered as a second-line drug in combination with other antiretroviral agents according to the World Health Organization guidelines (2). The most important adverse effect of ABC that limits its usage is an immunologically mediated hypersensitivity reaction that occurs in 5% - 8% of Caucasian patients receiving highly active antiretroviral therapy (HAART) (1, 3). ABC HSR is a life threatening clinical syndrome that affects multiple organ systems; it is characterized by constitutional symptoms (malaise,

headache, and dizziness), fever, rash, gastrointestinal symptoms (nausea, vomiting, and diarrhea), and respiratory symptoms (dyspnea, and cough). The nonspecific symptoms of HSR may lead to clinical over-diagnosis, which may prevent patients from receiving ABC containing regimen (4).

The relationship between HLA class I allele, HLA-B*5701, and ABC HSR was first reported by Mallal et al. (5) in Australian patients. Subsequent studies showed that genetic susceptibility to ABC HSR is strongly associated with the presence of HLA-B*5701 in different populations (4). While the frequency of HLA-B*5701 has been reported in different ethnic groups (6.5% in Caucasians, 1% in sub-Saharan African, 5% to 20% in India, 4% to 10% in Thailand, 1% to 2% in the Mediterranean and ~0% in Korea), there are few reports from the Middle Eastern countries that estimate the prevalence of this allele in healthy patients (2.7% in Morocco, and 1% in Jordan) (6-11). It has been shown that screening for HLA-B*5701 could significantly reduce the incidence of ABC HSR (6).

To our knowledge, there are no data on the prevalence of HLA-B*5701 allele in Iranian HIV positive patients. Therefore, our goal was to assess the prevalence of HLA-B*5701 in our patients and evaluate its relationship with ABC HSR.

MATERIALS AND METHODS

Study design

From February 2007 to January 2013, blood samples were collected from HIV positive patients (diagnosis confirmed by HIV antibody testing and molecular diagnosis). Hypersensitivity reaction to ABC was investigated in the patients who had received ABC therapy. Patient demographics, HLA typing, CD4 lymphocyte count, concurrent medication usage, history of food and drug allergies, and the date of ABC discontinuation were recorded. Clinical diagnosis of ABC HSR was made based on the appearance of symptoms within 6 weeks of ABC initiation and disappearance within 72 hours of ABC discontinuation. The symptoms included fever and constitutional symptoms (e.g., malaise, dizziness, and headache), gastrointestinal disturbances (e.g., nausea, vomiting, and diarrhea), and respiratory symptoms (e.g., dyspnea, and cough) (12). The study protocol was approved by the ethics committee of the National Research Institute of Tuberculosis and Lung Disease.

Sample size calculation

Caucasians have the closest ethnicity to Iranians (13) and our sample size was calculated using the prevalence of HLA-B*5701 allele in Caucasian patients of European heritage (14). The minimum number of subjects required to estimate the frequency of the allele was calculated to be 176 patients.

DNA extraction and HLA typing

DNA from blood samples was prepared according to the methods described in the High Pure Viral Nucleic Acid Kit (Roche, Germany). The quality of the extracted genome in the specimen for PCR assay was evaluated by the detection of the beta-globin housekeeping gene using the GH20/PC04 primer set (15).

HLA typing was carried out according to previously described methods (16). The primers used in the study were as follows: Primer 1F: 5'-GTCTCACATCATCCAGGT-3', primer 2R: 5'-ATCCTTGCCGTCGTAGGCGG-3', primer 3R: 5'-ATCCTTGCCGTCGTAGGCAG-3'. The SYBRgreen real-time PCR was performed in 25 µl reaction volume using Maxima SYBRgreen q PCR MasterMix (ThermoScientific, USA), 0.04 µM for each primer, and 5 µl of extracted genome.

PCR and melting curve analysis were performed via CFX 96 Bio-Rad instrumentation. PCR was carried out as follows: Initial denaturation 95 ^{o}C for 10 minutes, 40 cycles; 95 ^{o}C for 5 seconds and 60 ^{o}C for 1 minute and the melting curve analysis was carried out at annealing temperature of 65 ^{o}C for 5 seconds followed by a gradual increase of temperature to 95 ^{o}C. The value for the respective melting temperature of c HLA-B*57:01 PCR product was 84 ^{o}C.

Statistical analysis

Descriptive statistics were performed to detail the demographic and clinical characteristics of the patients. Non-normally distributed variables were reported as medians. Fisher's Exact Test was used to assess the relationship of ABC HSR with HLA-B*5701. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 19.0.

RESULTS

Of the 198 blood samples that underwent HLA typing, six samples (3.0%) were positive for HLA-B*57:01 allele (95% CI, 1%–5%). Demographic and clinical characteristics of the patients are presented in table 1. ABC was administered to 28 patients. Table 2 summarizes demographic and clinical characteristics of these patients. Of the four patients with suspected ABC HSR, two were excluded because their reactions were thought to be caused by concurrent medication administration according to Naranjo ADR Probability Scale (17). The third patient, who had an incomplete drug history, was also excluded. Only one patient that experienced HSR based on Naranjo ADR Probability Scale was HLA-B*5701 carrier. There was no relationship between the history of allergy and ABC HSR. Fisher's exact test showed a relationship between HSR and HLA-B*5701 (P=0.036).

Table 1. Demographic and clinical characteristics of 198 patients

Variables		Data
Sex	Male, n (%)	172 (86.9)
	Female, n (%)	26 (13.1)
Age, median (range)		37 (1-87)
CD4⁺ cell count, median cells/µl (range)		77.5 (2-800)
HLA-B*5701 status, n (%)	Positive	6 (3)
	Negative	192 (97)
Patients on HAART, n (%)	No	77 (38.9)
	Yes	121 (61.1)
Patients on ABC, n (%)	No	170 (85.8)
	Yes	28 (14.1)

Table 2. Demographic and clinical characteristics of 28 patients on ABC

Variables		Data
Sex	Male, n (%)	25 (89.3)
	Female, n (%)	3 (10.7)
Age, median (range)		38 (20-57)
CD4⁺ cell count, median cells/µl (range)		55(4.3-680)
HLA-B*5701 status, n (%)	Positive	1 (3.6)
	Negative	27 (96.4)
Previous antiretroviral drug therapy, n (%)	No	2 (7.4)
	Yes	26 (92.6)
History of allergy, n (%)	Yes	1 (3.6)
	No	24 (85.7)

DISCUSSION

The prevalence of HLA-B*5701 allele in our patient population was 3.0% (95%CI: 0.01-0.05). The prevalence of this allele been widely studied in deferent geographical areas, races, and ethnicities and varies significantly between different. The reported prevalence may be affected by the sample size and ethnic composition of the population (6,18). The difference between the prevalence of this allele in our sample and that reported in Caucasians might be due to our limited sample size, or the ethinc differences between Iranians and Caucasians. Generally, the prevalence of carriers has been reported to be 6.8% in Europeans, 2.6% in South Americans, 1.0% in Africans, 2.5% in Middle Easterns, 2.2% in Mexicans, 1.6% in Asians, and 11% in Southwest Asians (19). The reported values in Asians and other Middle Eastern populations are comparable with our results (6,19).

We found a relationship between HSR and HLA-B*5701 (P<0.05), which are consistent with reports by other investigators (20-22). ABC was discontinued in four patients with suspected HSR, while one of them had a confirmed reaction. The symptoms of ABC HSR are non-specific and may be misdiagnosed as viral infection or reaction to other drugs (4, 12). Misdiagnosis of HSR may prevent patients from receiving appropriate treatment containing ABC. Our data shows that HLA-B*5701 screening may help not only to reduce the risk of HSR but also to prevent the clinical over-diagnosis.

Screening for HLA-B*5701 in all abacavir-naive individuals before initiation of abacavir-containing therapy has been recommended by the Clinical Pharmacogenetics Implementation Consortium Guidelines. ABC should not be administered in HLA-B*5701 positive patients. On the other hand, a negative result for HLA-B*5701 cannot reliably exclude the possibility of an ABC HSR and patients should be monitored continuously while receiving this medication (6,19). Therefore, accurate reporting of the HLAB*5701 allele could reduce the risk of ABC HSR.

In addition to reliable laboratories required for implementation of the routine screening, the cost-effectiveness of this pharmacogenetic test should be evaluated. The cost-effectiveness of HLA-B*5701 genotyping has been shown in different countries such as the UK (23), Spain (24), France (25), and the USA (26). However, the structure of health care system in a country is a determining factor for health economic evaluation. Our results could be used to design a cost-effectiveness study for HLA-B*5701 screening in our country.

CONCLUSION

The prevalence of HLA-B*5701 in our HIV positive patients is lower than that in Caucasians but comparable with that in other Middle Eastern populations. Pretreatment screening may reduce the incidence of ABC HSR, as well as its clinical over-diagnosis. However, economic issues should be considered. We suggest future economic feasibility studies to determine the necessity of this test in Iranian patients.

Acknowledgments

The authors would like to acknowledge the assistance of Dr. Haleh Rezaie and the support from Afsaneh Sigaroodi, Leila Poosashkan, Mojgan Mohammadi, Ghazal Bagheri and Negah Bamdadian.

Conflict of interest

Authors have no conflicts of interest to declare.

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