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MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

PHARMACOGENETICS TESTING FOR HLA-B*57:01 ALLELE BY SCREENING ASSAYS AND HIGH RESOLUTION HLA-TYPING: RESULTS FROM A COMPLETE NATIONAL HIV COHORT, CROATIA

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Abstract: The HLA-B*57:01 allele is associated with hypersensitivity to the antiretroviral drug abacavir. Current HIV treatment guidelines recommend screening for HLA-B*57:01 before initiation of abacavir-containing regimen. The aim of this study was to determine the prevalence of HLA-B*57:01 in the Croatian HIV cohort. The study included all HIV-infected individuals receiving clinical care at the Croatian Reference center for HIV/AIDS, University Hospital for Infectious Diseases, Zagreb, Croatia in the period 2009-2017, 1288 in total. HLA-B*57:01 typing was performed by using PCR:SSO reverse hybridization method and PCR-SSP high resolution typing only for ambiguous HLA-B*57:01/*57:06 samples in the period 2009-2012, while starting from January 2013 the typing method was switched to Real-Time PCR. HLA-B*57:01 allele was detected in 65 of 1288 (5.1%) HIV-infected individuals, revealing the HLA-B*57:01 allele frequency of 2.5%. The HLA B*57:01 frequency in positive HIV infected male individuals (2.6%) was concordant with the frequency in the general Croatian population, as well as with the frequency in the entire group of HIV-positive individuals from Croatia, while HIV infected women showed somehow lower frequency of HLA B*57:01 allele (1.5%). No clinical events associated with abacavir hypersensitivity have been observed since the introduction of the prospective HLA-B*57:01 screening, while two non-fatal cases of hypersensitivity were observed prior to introduction of the screening.

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Submitted: November, 2017
Accepted: December, 2017

Key words: abacavir, HLA B*57:01 allele, HIV infection, hypersensitivity reaction

INTRODUCTION

The human leukocyte antigen (HLA) allele, HLA-B*57:01 is associated with hypersensitivity to the antiretroviral drug abacavir, a nucleoside reverse transcriptase inhibitor (NRTI) approved for use in combination with other antiretroviral drugs for treatment of human immunodeficiency virus (HIV) infection. A large number of clinical studies confirmed clinical efficiency of abacavir as a part of highly active antiretroviral therapy (HAART), few drug interactions and favorable long-term toxicity profile.[1, 2, 3]

However, a possibility of life-threatening adverse events associated with hypersensitivity to abacavir requires careful clinical follow-up of patients. Symptoms of the hypersensitivity to abacavir are non-specific and include: fever, rash, gastrointestinal, constitutional and respiratory symptoms that are usually reversible upon discontinuation of abacavir. Continuation of abacavir use after the hypersensitivity reaction as well as re-challenge with the drug are contraindicated due to the possibility of life-threatening reactions.[4] An association between hypersensitivity reaction to abacavir and HLA-B*57:01 allele was first reported in 2002 by two independent research groups[1, 2] and later confirmed by other studies as well.[5, 6, 7] In 2008, a double-blind, prospective, randomized clinical trial PREDICT-1 (Prospective Randomised Evaluation of DNA Screening in a Clinical Trial) that included 1956 mostly individuals of European origin HIV-infected patients from 19 countries confirmed that prospective HLA-B*57:01 screening reduced the risk of hypersensitivity to abacavir. The prevalence of HLA-B*57:01 allele in the cohort (predominantly individuals of European origin) was 5.6%.[3] Prospective screening for HLA-B*57:01 in this cohort eliminated

abacavir hypersensitivity compared with patients that were not screened for this allele prior to abacavir initiation (0% vs. 2.7%). Current European and international HIV treatment guidelines recommend screening for HLA B*57:01 before initiation of abacavir-containing regimen.[8, 9]

HLA B*57:01 allele is also more frequently found in HIV slow progressors or non-progressors. Persons carrying this allele may have higher CD4+ counts, lower viral load and better response to HAART. Proposed mechanism of action is through more effective T-cell responses to HIV antigens in HLA B*57:01 positive individuals.[10, 11]

Croatia has low-level HIV epidemics and centralized system of diagnostics and care at the University Hospital for Infectious Diseases (UHID). In the period from1985 till October 2017, the cumulative number of HIV infections was 1403, with a total of 202 deaths.[12]

The aim of this study was to determine the prevalence of HLA-B*57:01 in the Croatian HIV cohort and to compare the results of the screening HLA-B*57:01 assay performed at the clinical molecular laboratory with high resolution HLA typing assay performed at the national reference Tissue Typing Center at University Hospital Center, Zagreb. To our knowledge, this study represents the highest proportion of the entire population of HIV-infected patients tested for HLA-B*57:01 in a single country.

MATERIALS AND METHODS

The study included all HIV-infected individuals entering clinical care at the Croatian Reference center for HIV/AIDS, University Hospital for Infectious Diseases, Zagreb, Croatia in the period between January 2009 to July 2017, 1288 in total. Peripheral blood samples were collected into K3EDTA tubes and stored at room temperature for flow cytometry analysis or at -20°C for HLA B*57:01 allele detection. DNA was isolated by using QIAamp DNA blood Kit (Qiagen, Hilden, Germany). HLA-B*57:01 detection was performed by Inno-LiPA HLA-B Multiplex Plus kit and INNO LiPA Update Plus kit, and analyzed using LiPA interpretation software (LiRAS^{TM}), (Innogenetics, Gent, Belgium) in the period from January 2009 till December 2012 and 810 individuals was tested using this method. All samples (n=22) with the Inno-LiPA assay test results including HLA-B*57:01/*57:06 ambiguities, were further analyzed by polymerase chain reaction-sequence specific priming (PCR-SSP) high resolution typing (Olerup SSP®, Sweden) at the Tissue Typing Center, University Hospital Center Zagreb, Croatia. Since January 2013, a 478 individuals was tested using Real-Time PCR test HLA B*57:01 Real-TM (Sacace Biotehnologies, Como, Italy).

RESULTS

The majority of enrolled patients were males (n=1157, 89.8%), whereas women represented only 10.2% of tested patients (n=131).

HLA-B*57:01 allele was detected in 65 out of 1288 (5.1%) of HIV-infected individuals, 61 males and 4 females. The analysis showed that 5.3% (61/1157) of HIV-positive men and 3.1% (4/131) of HIV-positive women were positive for HLA-B*57:01 allele (Table 1). Furthermore, the study showed the HLA-B*57:01 allele frequency of 2.5% in the entire group of HIV infected patients and 2.6% in HIV positive males, whereas the allele frequency in female patients was somehow lower (1.5%), although the difference did not reach statistical significance.

No clinical events associated with abacavir hypersensitivity have been observed since the introduction of the prospective HLA-B*57:01 screening, whereas prior to the introduction of screening two non-fatal cases of abacavir hypersensitivity in the cohort were observed.

Table 1. Number of B*57:01-positive samples among all patients and among patients divided by gender

	Total N (%)	B*57:01-positive N (%)
Total	1288	65 (5.0)
Males	1157 (89.8)	61 (5.3)
Females	131 (10.2)	4 (3.0)

DISCUSSION

The clinical usefulness of pharmacogenetic screening for HLA-B*57:01 in reducing the likelihood of hypersensitivity to abacavir has been clearly demonstrated in a PREDICT-1 clinical trial.[3] Both European AIDS Clinical Society and Department of Health and Human Services Panel Guidelines on diagnostics and treatment of HIV-infected patients recommend HLA-B*57:01 genetic assay as a part of the diagnostic work-up of all HIV-infected patients during their first visit to the clinician following the diagnosis.

Our results comparing the results of the HLA-B*57:01 screening assays and the results of HLA*57:01 allele high resolution typing were completely concordant demonstrating the suitability of screening assays for routine diagnostic use.

However, experts have emphasized that the clinical utility and cost-effectiveness of genetic screening for abacavir hypersensitivity depend on the prevalence of HLA-B*57:01 in a local population that needs to be investigated in details.[14]

The results of this study showed that the prevalence of HLA-B*57:01 carriage in Croatian HIV-infected patients was consistent with other studies reporting prevalence in individuals of European origin HIV-

infected patients from Europe, Australia and the USA ranging between 5.0% to 8.0%.[14-16]

Literature data on the prevalence of HLA-B*57:01 in HIV-infected patients and general population worldwide have shown extensive heterogeneity associated with ethnicity.

The highest prevalence of HLA-B*57:01 was observed in individuals of European origin. A largest European study that included 9720 HIV-infected patients from 10 countries showed 6.5% prevalence of HLA-B*57:01 in self-reported individuals of European origin.[17] Arrizabalaga et al (2009) conducted one of the largest national cross-sectional studies on the prevalence of HLA-B*57:01 in HIV-infected patients that included 1198 patients from 74 HIV-clinical centers across Spain.[18] The prevalence of HLA-B*57:01 in individuals of European origin subgroup of patients (92.2% of total study population) was estimated at 6.5%. Similar results were obtained in two recent studies on the prevalence of HLA-B*57:01 in individuals of European origin conducted in Poland (4.7%) and Georgia (5.6%).[15, 19] Western Australian HIV Cohort Study on the prospective genetic screening for abacavir hypersensitivity showed 7.7% prevalence of HLA-B*57:01 in Caucasian subgroup of patients[16]

Orkin et al (2010) performed an extensive analysis of HLA-B*57:01 prevalence in the group of 1494 HIV-1 infected patient from 8 clinical centers at the United Kingdom as well as in two major subgroups (individuals of European and African origin).[17] The prevalence of HLA-B*57:01 among white subjects was higher (7.9%) compared to black subjects of mostly African origin (0.2%) as well as overall prevalence in the study group (4.5%).

Very low prevalence of HLA-B*57:01 allele in African HIV-infected patients was confirmed by the recent results of the NORA pharmacogenetic sub-study of DART conducted in Uganda.[20] HLA-B57:01 allele was absent from the cohort of 247 patients receiving abacavir but clinically diagnosed hypersensitivity reaction to abacavir was detected in 2% of patients. Due to the fact that no HLA-B*57:01 alleles were found in the abacavir-treated group, clinically diagnosed hypersensitivity reactions were presumed to be false-positive as expected from previous clinical studies.

The data on the prevalence of HLA-B*57:01 in HIV-infected patients from Latin American countries are scarce. Poggi et al (2010) recently reported 2.2% carrier frequency in HIV-infected patients from Chile and 3.7% frequency in general population.[21]

Studies in individuals of Asian origin from East Asia (Taiwanese, Japanese, Chinese and Korean) reported very low prevalence of HIV-B*57:01.[22-25] Only one of 337 (0.3%) HIV-infected Taiwanese patients expressed HLA-B*57:01 allele whereas the most common allele reported in the study was HLA-A*02.[23] A study conducted in Japan (n=371) reported 0% of HLA-B*57:01-positive individuals.[24] Similarly, the prevalence of HLA-B*57:01 in Hong Kong Chinese patients (n=572) was only 0.003%.[24] These studies raised a question on the usefulness and cost-effectiveness of routine HLA-B*57:01 screening in populations of individuals of Asian origin with very low prevalence of HLA-B*57:01.[25] However, it should be emphasized that even in countries with low prevalence of HLA-B*57:01 the screening can be a useful in reducing clinical over-diagnosis of hypersensitivity reaction to abacavir.

In this study, the HLA B*57:01 frequency in positive HIV infected male individuals was concordant with the frequency in the general Croatian population[26], as well as with the frequency in the entire group of HIV-positive individuals from Croatia, while HIV infected women showed somehow lower frequency of HLA B*57:01 allele, without reaching the statistically significant difference. One of the probable explanations may be found in the size difference between the groups, although all groups were large enough to be included in HLA allele frequency analysis. Thus, it will be of interest to perform in future further investigation the HLA B*57:01 allele frequency in the larger group of female HIV infected patients.

In conclusion, the prevalence of HLA-B*57:01 in the HIV infected Croatian national cohort was in accordance with the reported literature data for Croatian healthy control group, as well as for HIV-infected patients from other populations of European origin. Introduction of prospective screening prevented abacavir hypersensitivity in the real-life clinical setting.

ACKNOWLEDGEMENT

This study was in part supported by Croatian Science Foundation grant: Molecular, epidemiological and clinical characteristics of HIV infection in Croatia (principal investigator Prof. Josip Begovac).

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