Prevalence of HLA-B*57:01 allele in HIV-positive and HIV-negative population of eastern India: An epidemiological study

Abhilasha Gautam a,1, Jaya Chakravarty a,x,1, Ankita Chourasia a, Saurabh Sharma a, Tanmoy Sarkar b, Parimal Das b

a Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, India
b Centre for Genetic Disorders, Faculty of Science, Banaras Hindu University, Varanasi, 221005, India

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A B S T R A C T

Introduction: The nucleoside analogue reverse transcriptase inhibitor Abacavir has potent antiviral activity against HIV; however, 5–8% patients develop hypersensitivity reactions within six weeks of treatment. The prevalence of the HLA-B*57:01 allele is strongly associated with the risk of Abacavir-associated hypersensitivity reaction (ABC-HSR). Prevalence of HLA-B*57:01 allele varies in different populations. This observational study was performed to determine the prevalence of HLA-B*57:01 allele in the population of Eastern India which included both HIV-positive and HIV-negative subjects.

Method: We screened 406 subjects attending the ART (antiretroviral treatment) centre and linked ICTC (Integrated counselling and testing centre), however 6 samples did not have adequate DNA. HLA-B*57:01 typing was done using direct sequence specific primer PCR (SSP-PCR). All PCR positive samples were sequenced using Sanger’s method.

Results: The prevalence of HLA-B*57:01 genotype in our cohort was 12.25% (49/400). Prevalence among men was 13.3% (33/248) and female was 10.5% (16/152). The prevalence was similar in HIV-positive subjects (13.5%) and in HIV-negative subjects (11%).

Conclusions: Prevalence of HLA-B*57:01 in our eastern Indian population was high. Therefore, screening for HLA-B*57:01 before ABC administration would be useful to prevent ABC-HSR.

1. Introduction

The National AIDS control program was providing free Antiretroviral therapy to 9,97,000 adult PLHIV (people living with HIV) and 55,606 CLHIV (children living with HIV) till September 2016. The National program has implemented the “Treat All” strategy, which recommends starting treatment for all PLHIV & CLHIV which will increase the number of PLHIV/CLHIV on treatment many folds. Abacavir (ABC) a guanosine analogue reverse transcriptase inhibitor is currently recommended as a part of first line regimen by National AIDS control Organization (NACO), India for children with haemoglobin <9 g/dl and as an alternate first line regimen for adults who have adverse effects with Tenofovir. It is an excellent drug in term of efficacy however, can cause a potentially life-threatening hypersensitivity reaction (HSR) in 5–8% patients within 4–6 weeks of treatment. Studies have shown a strong association between ABC-HSR and the major histocompatibility (MHC), HLA class I allele, HLA-B*57:01. To avoid this HSR, routine screening for HLA-B*57:01 is recommended in resource rich setting before start of therapy and abacavir is only prescribed if a patient tests negative.

The prevalence of HLA-B*57:01 allele varies among different ethnicity. Among Caucasians, the prevalence was 7.93% whereas among those of African origin only 0.26%. The reported prevalence of HLA-B*57:01 in East Asian populations was lower than 0.3% among Koreans and Taiwanese, 3.4–4% in children from Thailand and Cambodia. The prevalence of this allele varies between 1.92 - 11% in various studies from India. While it is 1.92% among North Indian Hindus, 5.33–5.91% in Western India, 8.2% among Nadar, Tamilians and 3.19% in other South Indians and 11% in HIV positive children from Mumbai. At present there are no studies on the prevalence of this allele in eastern India. Thus, the main objective of the study was to

1 Corresponding author. Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, India.
E-mail addresses: abhilasha.kumaon@gmail.com (A. Gautam), tapadar@gmail.com (J. Chakravarty), ankita.chourasia21@gmail.com (A. Chourasia), saurabh3b@gmail.com (S. Sharma), tanmoy258819@yahoo.co.in (T. Sarkar), hellow_parimal@yahoo.com (P. Das).
Joint First author.

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determine the prevalence of HLA-B*57:01 allele in population of Eastern India which included both HIV-positive and HIV-negative subjects.

2. Methods and material

2.1. Study settings, design, period & population

This observational study was conducted between 2013 and 2016 in the Antiretroviral Therapy, (ART) Centre of Department of Medicine, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), India. The source population were all HIV-positive adults attending ART centre who were fulfilling the inclusion criteria. The participants were from 15 districts of Eastern Uttar Pradesh.

Ambedkar nagar (6), Allahabad (5), Azamgarh (51), Ballia (42), Bhadoi (17), Chandoli (34), Deoria (11), Ghazipur (28), Gorakhpur (2), Jaunpur (37), Mau (31), Mirzapur (38), Pratapgarh (1), Sonbhadra (19), Varanasi (78).

2.2. Inclusion and exclusion criteria

Inclusion Criteria.
1. Participate above the age of 18 years.
2. Residing in eastern Uttar Pradesh and willing to participate in the study.

2.3. Sample size determination and sampling technique

As the prevalence of HLA-B*57:01 allele in different Indian population varied from 1.92-8.2% we assumed 4% prevalence of this allele in eastern Indian population having similar ethnic characteristics which included both HIV positive and negative adults as the HLA-B*57:01 allele doesn’t vary with HIV status. Using 2% precision, the sample size was calculated by the formula \( n = \left( \frac{z}{p} \right)^2 \frac{q}{d^2} \) where \( n \) = sample size, \( z \) = level of confidence according to the standard normal distribution (for a level of confidence of 95%, \( z = 1.96, p = \) estimated proportion of the population that presents the characteristic \( (p = 0.04) \) assumed that the prevalence is same in both HIV positive and negative adults), \( d = \) tolerated margin of error. The sample size was calculated to be 369 subjects. Considering, the 10% wastage of samples, it was increased to 406 subjects. A list (sampling frame) was prepared of HIV-positive adults attending ART centre and subjects testing HIV-negative in the linked ICTC centre.

2.4. Data collection tools & procedures

Baseline data of CD4 count, WHO stage and opportunistic infection of the HIV-positive participants were taken from database of the ART centre. Demographic data were collected for HIV-negative adults participating in the study in the ART centre. CD4+ lymphocyte counts (BD FACS Calibur) were available only for HIV-positive subjects as it is done routinely as per NACO guidelines and the values were expressed as cells/\( \mu l \).

2.5. Sample collection and DNA extraction

Whole blood samples from each subject (2.5 ml) were collected in an EDTA vial. Separation ofuffy coat from whole blood was done by centrifugation at 2500 rpm for 10 minutes at 4 °C. Buffy coat was transferred in to a sterile polypropylene tube and stored at –20 °C till DNA extraction. DNA was extracted from the buffy coat using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer’s protocol and stored at –20 °C until further processing. The quality and quantity of DNA was checked by agarose gel electrophoresis and NanoDrop (Thermo Scientific, USA).

2.6. HLA B*57 allele specific amplification by PCR

In the HLA B*57 database, that highest variability in SNPs is observed in the exon 2 and exon 3 region which are peptide-binding domains of the MHC class I molecule. Therefore, we selected primer targeting exon-3 region for high resolution HLA B*57 typing. HLA B*57 exon 3 region was amplified by sequence-specific primer (SSP). The primers used for HLA B*57 amplification in the study were as follows: Primer F: 5′-GTCCATCATCTCAGG-3′ and primer R: 5′-GAGCTTCATTGGCGTGGCT-3′ (Eurofins Genomics India Pvt Ltd) which amplifies a 262bp amplicon. The reaction mixture was prepared in a total volume 25 μl containing 2 μl of the extracted DNA (50 ng/μl) along with 12.5 μl Dream Taq Green PCR master mix (Thermo Scientific, USA) and the primers 1 μl each (10 pmol/μl each). DNase/RNase free water was used to make up the final volume. The presence of human genomic DNA was evaluated by Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene as internal control. The primers used for GAPDH amplification as follows: Primer GAPDH-F (5′-GCCACGCAACAGGATCTTTAC-3′) and primer GAPDH-R (5′-GCCACACACCCTGGTC-3′) which amplifies a 136bp amplicon. The mixture was subjected to 35 cycles of amplifications using Veriti 96 Well Thermal Cycler (Applied Biosystems, USA). Each cycle included a denaturation step at 94 °C for 30 seconds, an annealing at 55 °C for 45 seconds, and a chain elongation step at 72 °C for 30 s. PCR products were then electrophoresed on 1.4% agarose gel stained with GoodViewTM Nucleic Acid stain, and amplicons were viewed and photographed by using the Gel Doc 2000 gel documentation system (Bio-Rad) under UV light using Alpha Innotech Gel documentation system.

2.7. HLA-B*57:01 sequencing

The samples positive by direct PCR were subsequently sequenced using Sanger’s method after DNA extraction from the agarose gel in order to establish the correct genotype. After kit-based purification, DNA sequencing was performed on conventional ABI 3130 with the same primers used for HLA B*57 amplification, at Centre for Genetic Disorders (CGD) at Faculty of Science, BHU. Data was analysed first in finchTV Software for SNP detection and then matched with IMGT (HLA-B*57) data Clustal W software for multiple alignment.

Reference Sequences IMGT: HLA-B*57:01data (Exon 3) GTTCTCA-CATCATCCAGTGTAGTATGCTGCGACCTGGGCGGCGGAGGCAGGACCCTCGCTCGCGGATTGACGGCTCCGGCTGAGTACAGTCCGCTTACGACGG-CAGAGGATTA-CATCGCCCTGAAAGGAGCCCTGAGCTGGAGGCGGGGCAAGC-GCCGAGGCCGCTCA-AGATC-CTGCGCAGTGCTGAGCGGGCGGCGGGGAA AGGGCGGCTGGTCA-GATCACCCAGCGCAAGTGGGAGGCGGCCCGTGTGGCGGAGCAGCTGA-GAGCCCTAAGGGGCGGCTGCGGG.

2.8. Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 16. The proportions were compared using Chi-square test or Fisher’s Exact test. We used t-test for parametric data and Mann–Whitney test for non-parametric data. The prevalence of the allele in HIV positive and negative participants was compared using 2-proportions test.

3. Results

3.1. Sociodemographic and clinical characteristics

We screened 406 HIV-positive and HIV-negative subjects from 15
Table 1
Baseline characteristics of HIV-positive and HIV-negative subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV-positive (N = 200)</th>
<th>HIV-negative (N = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA-B'57:01 Positive carrier (n = 27)</td>
<td>HLA-B'57:01 Negative Carrier (n = 173)</td>
</tr>
<tr>
<td>Age in years Median (IQR)</td>
<td>36 (28-45)</td>
<td>35 (30-40)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 20 (74.1%)</td>
<td>102 (59.0%)</td>
</tr>
<tr>
<td></td>
<td>Female 7 (25.9%)</td>
<td>71 (41.0%)</td>
</tr>
<tr>
<td>CD4 count(ul) Median (IQR)</td>
<td>168 (95-268)</td>
<td>150 (67-244)</td>
</tr>
<tr>
<td>WHO Stage</td>
<td>I 15 (57.7%)</td>
<td>106 (61.3%)</td>
</tr>
<tr>
<td></td>
<td>II 0 (0%)</td>
<td>12 (6.9%)</td>
</tr>
<tr>
<td></td>
<td>III 8 (30.8%)</td>
<td>26 (15%)</td>
</tr>
<tr>
<td></td>
<td>IV 3 (11.5%)</td>
<td>29 (16.8%)</td>
</tr>
<tr>
<td>Duration of ART Median (month)</td>
<td>44 (18-77)</td>
<td>38 (22-63)</td>
</tr>
</tbody>
</table>

¹ Chi-square.
² Mann whitney.

Fig. 1. Gel electrophoresis of direct HLA-B57:01 products M: ladder (50bp), Lane 2–11; B57* positive and B57* negative samples (262bp), GAPDH (136bp) and Negative control (NC).

Fig. 2. Output of Sequence in FinchTV Software shows chromatogram peaks of HLA- B*57:01 allele positive sample.
characteristics of all 400 subjects are given in (Table 1). Among the HIV-CD4 count were 151 cells/μl inadequate and the data presented here is of 400 participants. Baseline districts of Eastern India however in six samples, quality of DNA was Table 2

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will prevent unnecessary discontinuation of abacavir.

concomitant illness which can be easily confused with ABC-HSR and it of these 8 children with clinical Abacavir hypersensitivity had

abacavir HSR and the drug was stopped. On testing for HLA-B*57:01 in [7.9% (95% CI 3.5–15.0%)] children developed clinically diagnosed abacavir HSR and the drug was stopped. On testing for HLA-B*57:01 in these 8 children only 2 of them were HLA-B*57:01 allele positive. Four of these 8 children with clinical Abacavir hypersensitivity had coexisting illness which can be easily confused with ABC-HSR and it was suggested that in the absence of HLA-B*57:01 testing in the program, treating coexisting illness prior to starting therapy in children will prevent unnecessary discontinuation of abacavir. The other option is to provide other antiretroviral drugs e.g., pediatric tenofovir in the program as first line so that use of abacavir in children is minimized.

5. Conclusion

High prevalence of HLA-B*57:01 allele in our population highlights risks towards the Abacavir Hypersensitivity Reaction (ABC HSR). Therefore, HIV-infected patients should be continuously screened before starting Abacavir therapy.

5.1. Limitations

The limitation of our study is the use of sequence-based genotyping the current gold standard for detection of HLA-B*57:01 allele carriers. This method is labour intensive, requires specialized laboratories, thus limiting its use in a resource poor setting like India. The need of the hour is development of simpler methods of detection of this allele without the use of sequence-based genotyping which can be implemented in resource poor settings.

Ethical consideration

The study was approved from the institutional review ethics committee of the IMS, Banaras Hindu University, under Dean/2013-14/EC/356 and written informed consent was obtained from all the participants. Participants were assured that their confidentiality would be maintained both during and after the study. Information given was used only for the purpose of this study.

Availability of data and materials

Most of the data is included in the manuscript. Additional can be found from the corresponding author based on reasonable request.

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Declaration of competing interest

None declared.

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