International Journal of Research in Medical Sciences
Youssoufi F et al. Int J Res Med Sci. 2019 Apr;7(4):1354-1359
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20191352

Original Research Article

HLA-B*44 allele associated with clinical parameters in HIV-1 infected Moroccan cohort

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Received: 24 January 2019
Accepted: 01 March 2019

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ABSTRACT

Background: The human leukocyte antigen-B*44 (HLA-B*44) allele has been reported to have promising results in the control of human immunodeficiency virus-1 (HIV-1) infection and associated with protection against HIV-1 disease progression. In the Moroccan HIV-1 infected patients, the contribution of this allele has not been established. This study aimed to evaluate the distribution of HLA-B*44 allele among HIV-1-infected in Morocco. Additionally, investigate HLA-B*44 allele association with demographical and HIV clinical parameters.
Methods: One hundred and sixty-seven HIV-1 infected, antiretroviral naive individuals were enrolled in this study. The HLA-B*44 allele screening was performed using the PCR amplification.
Results: Of the 167 individuals genotyped, 26 (16%) of them expressing the HLA-B*44 allele. Clinical stages at diagnosis, median pre-treatment HIV viral load (pVL) and CD4 T cell counts differ significantly (p = 0.0001, p=0.001 and p=0.0001 respectively) between the patients who had been expressing the HLA-B*44 allele and patients who had not been expressing this allele. The presence of HLA-B*44 allele was significantly associated with pVL and CD4 T cell counts (p=0.004 and p=0.0001 respectively). The bivariate analysis has showed that the expression of the HLA-B*44 allele was strongly associated with advanced HIV infection (Odd ratio (OR) 0.12 (95% confidence interval (CI) 0.04-0.37), p=0.0001).
Conclusions: Author have described for the first time in Morocco the association of the HLA-B*44 allele with the clinical parameters of HIV infection. These results expand the knowledge of the distribution and effect of this allele in the Moroccan population.

Keywords: HLA-B*44 allele, HIV infection, Morocco

INTRODUCTION

The progression of human immunodeficiency virus (HIV) disease is highly variable among individuals and several factors are known to play a role in determining the rate of its progression. One of the important factors is host genetics, including the human leukocyte antigen (HLA) genes.[1,2] HLA class I have an important role in the innate and adaptive immune responses during the course of HIV-1 infection.[3-5] The HLA-B locus is the most HLA-I

polymorphic. Based on current estimates, there are over 4859 allelic variants of HLA-B.[3] HLA-B*35:02 and B*35:03 alleles in Caucasian patients have been associated with more rapid disease progression.[6,7]

Whereas, HLA-B*57, HLA-B*27 and HLA-B*44 alleles have been associated with slower disease progression.[8-11] HLA-B*44 has been reported to have promising results in the control of viremia during two distinct phases of primary HIV-1 infection in individuals in sub-Saharan Africa and in individuals in the United States with long-term HIV-1 infection.[10,12,13]

Based on this background, this study aimed to evaluate the distribution of HLA-B*44 allele among HIV-1-infected in Morocco. Additionally, author investigated HLA-B*44 allele association with demographical and HIV clinical parameters.

METHODS

Patients

This study was evaluated by the Ethics Committee for Biomedical Research in Rabat registered at the Office for Human Research Protections in US Department of Health and human Services under the number IORG0006594. All participants were adults (over 18 years) and gave written informed consent before blood sample donation.

One hundred and sixty-seven HIV-1 infected, antiretroviral naive individuals from the infectious diseases department of the Mohammed V Military Hospital of Rabat, Morocco were enrolled by convenience sampling from December 2014 and June 2016. Whole-blood samples were collected in EDTA treated tubes and cryopreserved until DNA extraction.

The following demographic and clinical data were collected which were age, gender, exposure category, pretreatment HIV viral load (pVL), baseline lymphocyte CD4 counts (first documented result after diagnosis of HIV) and clinical category at diagnosis according to WHO clinical classification of established HIV infection. The advanced HIV infection was defined according to the WHO clinical criteria for diagnosis of advanced HIV in adults (presumptive or definitive diagnosis of any stage 3 or stage 4 condition and/or CD4 count less than 350/mm^3 of blood in an HIV-infected adult).

HIV clinical parameters

HIV pVL was determined by automated real-time polymerase chain reaction (PCR) using the Cobas Ampliprep/Cobas TaqMan system (Roche Diagnostics, Mannheim, Germany) with a detection limit of 20 HIV RNA copies/mL. CD45+, CD3+, CD4+ and CD8+ cell counts were obtained by flow cytometry using the Navios Flow Cytometer (Beckman Coulter).

DNA extraction

Total DNA was prepared from whole-blood samples, using the Purelink genomic DNA Kit (Invitrogen, Life technology, USA) according to the manufacturer's instructions. Briefly, aliquots of 200 µl for each whole-blood sample were used and the total DNA was eluted in 100 µl elution buffer. The Qubit dsDNA HS (High Sensitivity) Assay kit was used to quantify the genomic DNA with the Qubit® Fluorometer (Invitrogen,Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's instructions.

*HLA-B*44 allele screening*

The HLA-B*44 allele screening was performed using the PCR amplification PCR as described previously.[14] Part of exon 3 of the HLA-B gene was amplified with oligonucleotides BX3S1: 5'-GGGTCCAGGGTCTCACATCA-3' and BX3R1: 5'-CCAGGTATCTGCGGAGCG-3' (Table 1).

The PCR reaction was performed in a total volume of 25 µl and consisting of 10 ng of genomic DNA, 5 pmol of each primer, 10X DNA polymerase PCR buffer (ABI buffer) added to a final concentration of 1X, 25 mM $MgCl_2$, 10 µmols of each dNTP (Life Technologies), and 0.4 µl of AmpliTaq (Biosystems).

A positive-control DNA (HLA-B*44 allele) was included in each amplification assay. As an internal control, 0.4 pmol human growth hormone (HGH) primers (HGH-I and HGH-II) amplifying a 439-bp fragment of the HGH gene were used to ascertain DNA quality and uniform assay condition.

Touch-down amplification-cycling conditions oniCycler® from BioRad (Hercules, Californie, USA), included at one cycle at 96ºC for 1 min, 10 cycles consisting of 30 s at 94ºC, 30 s at 65ºC and 60 s at 72ºC followed by 25 cycles consisting of 30 s at 94ºC, 30 s at 60ºC, 60 s at 72ºC. PCR products were electrophoresised on a 3% agarose gel (SIGMA, Saint Louis, USA). Results were visualized under UV light. The interpretation of the results was based on the presence or the absence of a specific amplified DNA fragment.

Statistical analysis

Descriptive statistics were achieved to detail the demographic and clinical characteristics of the patients. Quantitative variables were described using means and standard deviations (SD) in cases in which the underlying distribution was normal. The median was used for variables without normal distribution. Allele frequencies were estimated by direct genotypic counts and expressed as percentage. Statistical comparisons were performed using the Chi2 tests for nominal variables. Continuous variables were analyzed using the t-test for normally distributed ones, while U-Mann Whitney and ANOVA

tests were used for non-parametric statistics. Bivariate analysis was also performed using logistics regression models to estimate the factors associated with advanced HIV infection. All statistical analyses were performed using SPSS software version 13.0. All P-values were two-tailed and were considered significant at <0.05.

Table 1: Demographic and clinical characteristics of patients and distribution of HLA-B*44 allele.

Characteristics	N= 167 (100%)	HLA-B*44 allele		p-value
		Presence of allele N = 26 (16%)	Absence of allele N=141 (84%)	
Age, mean (SD)	43 (±11)	40 (±12)	44 (±11)	0.81
Gender				
Men	118 (70.7)	14 (8.7)	104 (62.0)	0.059
Women	49 (29.3)	12 (7.3)	37 (22.0)	
Exposure category				
Heterosexual	147 (88)	24 (14.7)	123 (73.3)	0.74
Other	20 (12)	2 (1.3)	18 (10.7)	
Clinical stages (WHO)*				
1	32 (19.1)	12 (7.1)	20 (12.0)	
2	47 (28.1)	10 (6.1)	37 (22.0)	0.0001
3	45 (27.0)	2 (1.4)	43 (25.6)	
4	43 (25.8)	2 (1.4)	41 (24.4)	
Plasma viral load (log copies/ml) Median (interquartile range)	5.14 (4.42-5.77)	4.93 (4.39-5.67)	5.42 (4.78-5.94)	0.001
<3	7 (4.3)	0 (0)	7 (4.3)	
3-5	73 (43.7)	19 (11.7)	54 (32.0)	
>5	87 (52)	7 (4.3)	80 (47.7)	
CD4 cell count (cells/µl) Median (interquartile range)	300 (160-473)	495 (367-600)	269 (141-408)	0.0001
<200	50 (30)	4 (3.0)	46 (27.0)	
200-350	46 (27.5)	2 (1.3)	44 (26.2)	
350-500	34 (20.4)	7 (4.3)	27 (16.1)	
>500	37 (22.1)	13 (7.4)	24 (14.7)	

* Stage 1: Asymptomatic, Stage 2: Mild symptoms, Stage 3: Advanced symptoms, Stage 4: Severe symptoms.

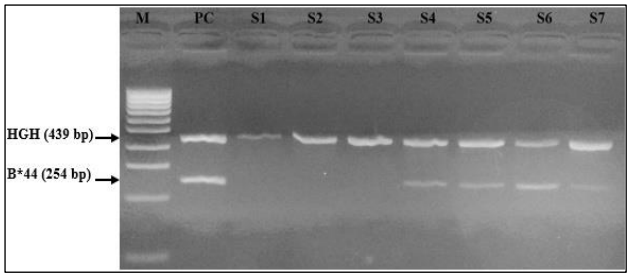
RESULTS

Cohort characteristics

The clinical and demographic characteristics of the 167 HIV-1 infected patients enrolled in this study are summarized in Table 1. The study has included patients with a median of 43 years (SD±11), with the majority being male (70.7%). The diagnosis of advanced HIV infection (stage 3 or stage 4) was in 52.8% of cases. The median of pVL was 5.14 (4.42-5.77) Log copies of HIV RNA/ml of plasma, while the median of CD4 count (before starting antiretroviral therapy) was 300 (160-473) cells/mm^3 (Table 1).

*Distribution of HLA-B*44 allele*

Of the 167 individuals genotyped, 26 (16%) of them had been expressing the HLA-B*44 allele. The amplification of HGH occurred in all samples with bands of 439 bp and the 254 bp product was detected in the samples carrying HLA-B*44 allele (Figure 1).



Lane 1,marker 100 bp; lane 2, positive control; lanes 3 to 9, patients (amplification of HGH occurred in all samples 439), the 254bp product was detected in patients expressing HLA-B*44).

Figure 1: Agarose gel electrophoresis illustrating PCR products for the HLA-B*44 allele.

Clinical stages at diagnosis, median pVL and CD4 T cell counts differ significantly (p=0.0001, p=0.001 and

p=0.0001 respectively) between the patients who had been expressing the HLA-B*44 allele and patients who had not been expressing this allele. Age, gender and exposure category did not differ significantly between the 2 groups (Table 1).

Association between the HLA-B*44 allele and clinical and demographic characteristics

Given the significantly difference between the patients who had been expressing the HLA-B*44 allele and patients who had not been expressing it, author explored the HLA-B*44 allele associations with HIV parameters. The Chi2 test has showed that the presence of HLA-B*44 allele was significantly associated with pVL and CD4 T cell counts (p=0.004 and p=0.0001 respectively). Author have also evaluated the factors associated with advanced HIV infection. The bivariate analysis showed that the expression of the HLA-B*44 allele was strongly associated with advanced HIV infection (Odd ratio (OR) 0.12 (95% confidence interval (CI) 0.04-0.37), p=0.0001) (Table 2).

Table 2: Bivariate analysis of the factors associated with advanced HIV infection.*

Characteristics	Bivariate analysis		
	OR	IC 95%	P-value
Gender			
Men	2.59	1.28-5.22	0.008
Women	1	-	-
Exposure category			
Heterosexual	0.59	0.19-1.86	0.37
Other	1	-	-
Plasma viral load			
<3	0.22	0.03-1.32	0.100
3-5	0.24	0.12-0.48	0.0001
>5	1	-	-
CD4 cell count			
<200	28.31	8.61-93.02	0.0001
200-350	10.35	3.50-30.62	0.0001
350-500	1.97	0.61-6.38	0.254
>500	1	-	-
HLA-B*44 allele			
Presence of allele	0.12	0.04-0.37	0.0001
Absence of allele	1	-	-

* Stage 3 or stage 4 condition and/or CD4 count less than 350/mm^3 of blood in an HIV-infected adult.

DISCUSSION

Several studies exhibited that susceptibility to HIV infection and the modulation of disease progression are strictly dependent on inter individual variability, much of which is secondary to host genetic heterogeneity.[15]

HLA system is considered as an important genetic factor that regulates the outcome of the infection. Generally, studies showed that HLA alleles such as, HLA-B*57, HLA-B*27 and HLA-B*44 are associated with resistance to HIV-1 infection and disease progression.[7,8,10,11] In this study, author have aimed to determine the frequency and the effect of HLA-B*44 against HIV disease in Moroccan HIV patients.

Author have investigated the frequency of HLA-B*44 allele in HIV-infected Moroccan cohort, 16% of the patients expressed HLA-B*44 allele. Few studies have been investigated HLA-B*44 allele in HIV-infected patients, because it has not been definitively associated with virology, immunologic, or clinical outcomes before, although one study has identified HLA-B*44:03 as a favorable allele in the context of HIV-1 subtype C infection in South Africa.[10,16] In 134 sub-Saharan seroconverts, only 12 (8.4%) subjects expressed HLA-B*4410.

In China, Zhang X et al, showed that 13 (10.3%) patients expressed HLA-B*44 allele in 126 Chinese patients with HIV-infection.[11] However, the frequency of this allele was similar in healthy subjects in the Mediterranean region. In Morocco, Spain, France and Tunisia the allele frequency in healthy persons was 12.4%, 15.4%, 16.2% and 11.79% respectively.[17-20]

In the present study, clinical parameters (Clinical stages, pVL and CD4 T cell counts) differ significantly (p=0.0001, p=0.001 and p=0.0001 respectively) between the patients who had been expressing the HLA-B*44 allele and patients had not been expressing this allele. In addition, the presence of HLA-B*44 allele was significantly associated with lower pVL and higher CD4 T cell counts (p=0.004 and p=0.0001 respectively).

Present results were consistent with the Sub-Saharan Africans results from 134 HIV-1, and with the Chinese results from 126 HIV-1 infected patients.[10-11]

Tang J et al, showed that in Sub-Saharan Africans HLA-B*44 allele was associated with lower VL (P=0.026) and higher CD4 counts (P= 0.022). Tang's study reported a significant association between HLA-B*44 allele and control of HIV-1 viremia during both the acute phase and the early chronic phase of the infection.[10]

Also, Zhang X et al, reported that patients expressing the HLA-B*44 allele showed significantly lower set point viral loads than those who did not express the B*44 allele.[11]

In this study, in bivariate analysis a significant association between HLA-B*44 allele and advanced HIV infection (OR 0.12 (95% CI 0.04-0.37) p=0.0001) has been found. The protective effect of this allele was reported in many studies.[21] This study has some limitations.

First, the patient population may not have been large enough to determine the influence of HLA-B*44 on

disease progression HIV-1 infection. Second, the difficult to establish a study cohort of seroconverts and to follow the clinical states of the patients, knowing that the results of the viral load and the CD4 cell counts allow, among other things, to evaluate the rate of progression of the infection.

CONCLUSION

This study corroborates the importance of the HLA-B alleles in determining the outcome of HIV infection. Here, author have described for the first time in Morocco the association of the HLA-B*44 allele with the clinical parameters of HIV infection.

These results expand the knowledge of the distribution and effect of this allele in the Moroccan population, where additional and large studies in the context of HIV infection are still needed.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee for Biomedical Research in Rabat (IORG0006594)

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Cite this article as: Youssoufi F, El Annaz H, Laraqui A, Tagajdid R, Abi R, Elkochri S, et al. HLA-B*44 allele associated with clinical parameters in HIV-1 infected Moroccan cohort. Int J Res Med Sci 2019;7:1354-9.