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Association Between Human Leukocyte Antigen Class-I and Hepatitis C: The First Report in Azeri Patients

Abolfazl Pourhassan
Department of Tropical and Infectious Diseases, Tabriz University of Medical Sciences, Imam Reza Hospital, Tabriz, Iran

Abstract: It has been suggested that host genetic diversity may be associated with Hepatitis C (HC). However, available data are tremendously heterogeneous due to the influence of ethnic and geographical differences. This study aimed to investigate possible association between certain Human Leukocyte Antigen (HLA) class-I alleles with HC in a group of Azeri patients for the first time in the literature. In a case-control study, 50 patients with confirmed HC (cases) and 50 healthy age- and sex-matched counterparts (controls) were evaluated in Tabriz Sina and Imam Reza Hospitals in a 2-year period of time (2011-2013). The investigated HLA alleles in the present study were: A_2, A_3, B_{35}, B_{38}, B_{W4}, C_{W4} and C_{W7}. The A_2-positive cases were significantly more frequent in the case than in the control group (58 vs. 32%, p = 0.01, Odds Ratio (OR) = 2.9). Similar trend was documented for A_3 (62 vs. 26%, p<0.001, OR = 4.6), B35 (24 vs. 2%, p = 0.001, OR = 15.5) and B_{W4} (78 vs. 46%, p = 0.001, OR = 4.2). In contrast, the rate of B_{38}-positive (34 vs. 8%, p = 0.001, OR = 0.2) and C_{W7}-positive (38 vs. 14%, p = 0.01, OR = 0.3) cases was significantly higher in the hepatitis-C-negative subjects. There was no significant difference in terms of the rate of C_{W4}-positivity between the two groups (20% in the cases vs. 34% in the controls, p = 0.12, OR = 0.5). This study showed that there are significant associations between certain HLA-I alleles with hepatitis C in Azeri patients. While some alleles make the host prone to the disease, others may have a protective role in this regard.

Key words: Hepatitis C, human leukocyte antigens class I, ethnicity, Azeri

INTRODUCTION

Hepatitis C (HC) is major health problem worldwide, particularly in developing countries in Asia and Africa (Hejazi *et al*., 2007; Bidgoli *et al*., 2007; Hemeida *et al*., 2011; Sohail *et al*., 2011; Kilic *et al*., 2012; Barakat *et al*., 2012; Abo Elmagd *et al*., 2011). The disease is caused by an enveloped blood-borne virus, which is known as the cause of post-transfusion non-A, non-B hepatitis. It is estimated the there are approximately 200 million individuals with chronic form of HC in the world. Although the average prevalence of the infection is rather high, it varies geographically from 0.1% in industrial countries to about 18% in Africa. The main route of transmission of HC virus is blood transfusion (Barth *et al*., 2006; Zeisel *et al*., 2009).

The extent of destruction induced by HC virus is determined by two factors; virus-related parameters such as heterogeneity, viral load and replicative potency and host-related parameters such as the efficacy of immune response (Chang, 2003). However, different clinical courses in similar patients with a single source of infection propose a possible role of host genetic factors (Sheehan *et al*., 1997). Human Leukocyte Antigen (HLA) is central to the host immune response and thus, is the nominee for investigating possible role of genetics in HC (Tripathy *et al*., 2009). Although there are numerous reports in this regard in the literature, the results are widely incongruous, presumably due to ethnic and geographical differences between studied populations (Wang *et al*., 2009; De Almeida *et al*., 2011). The objective of the present study was to investigate possible association between certain HLA class-I alleles and hepatitis C in a group of Azeri patients for the first time.

MATERIALS AND METHODS

In this case-control setting, 50 patients with hepatitis-C (the case group) and 50 healthy subjects (the control group) were recruited in a study during a 2-year period of time (2011-2013) in Tabriz Imam Reza and Sina Teaching Hospitals. The diagnosis of hepatitis-C infection was made by appropriate molecular and serological testing.

This study was approved by the ethics committee of Tabriz University of the Medical Sciences. Written consent was obtained from the participants.

The hepatitis-C infection was diagnosed by detection of anti Hepatitis C Virus (HCV) antibody using Enzyme Linked Immunosorbent Assay (ELISA) technique (Ortho Clinical diagnostics, Inc. 3rd generation, New Jersey, USA) and confirmed by immunoblot assay. The same method was employed for rolling out infection in the control group.

The control group was consisted of 50 age and sex-matched healthy individuals, who were selected from organ donating volunteers.

Subjects positive for hepatitis-B (positive HBsAg) or Human Immunodeficiency Virus (HIV) infections were not included. Both patients and controls had normal liver function tests at the time of enrollment.

The Human Leukocyte Antigens (HLA) evaluated in the present work were as follows; A_2, A_3, B_{35}, B_{38}, B_{W4}, C_{W4} and C_{W7}. For HLA typing, 10 mL peripheral blood was taken from the participants and its fibrinogen content was extracted. About 4 mL of this fibrinogen-free blood sample was mixed with 4 mL of Hank's solution and the new product was mixed gently with ficoll-hypaque solution (density = 1.077, ratio: 3/5 mL). After being centrifuged, lymphocytes were extracted and washed with Hank's solution three more times. After being centrifuged for three times the washed lymphocytes were placed into wells on a commercial 72-well class I typing trays (Biotest, Germany), previously coated with anti-HLA Class-I antibodies. After 30 min incubating on a shaker, exogenous (rabbit) complement was added into the plates. After 90 min shaking, eosin and formalin were added and after 24 h plates were examined by using a standard reverse microscope. After interpretation of the pattern of reactivity, the HLA typing was finalized (Ray, 1980). The rate of HLA positivity was compared between the case and control groups.

Statistical analysis: Data were shown as Mean±standard deviation or number (%). The SPSS software for Windows (ver.16) was used. Independent samples t test (for age) and the Chi-square test (for sex and the status of HLA) were employed for analyzing. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Fifty hepatitis-C positive patients, including 35 males (70%) and 15 females (30%) were compared with fifty hepatitis-C negative individuals, including 33 males (66%) and 17 females (34%). The two groups were comparable in terms of participants' gender (p = 0.69).

The mean age of the patients in the hepatitis-C positive group was 44.7±12.1 (range: 16-66) years vs. the mean age of 43.2±11.2 (range: 18-69) years in the healthy group. Age distribution of the participants in the two studied group is shown in Fig. 1. The two groups were comparable in terms of their participants' age (p = 0.47).

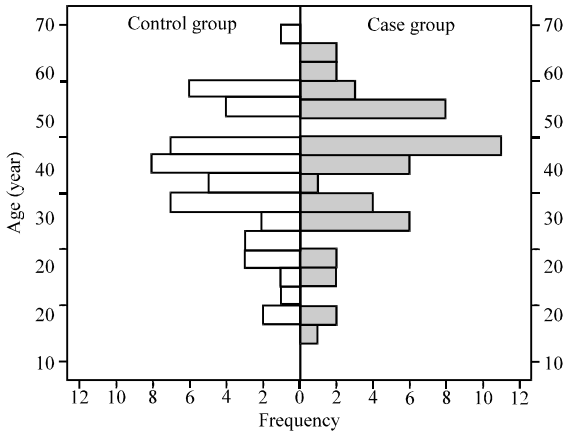

Fig. 1: Age distributions of the participants in hepatitis-C positive and negative groups

Table 1: Positive human leukocyte antigen class I (HLA_I) in hepatitis-C positive and negative subjects

	Hepatitis-C				
HLA-I	Positive	Negative	p-value	Odds ratio	95% CI
A_2	29 (58)	16 (32)	0.010	2.9	1.3-6.6
A_3	31 (62)	13 (26)	<0.001	4.6	2-10.9
B_{35}	12 (24)	1 (2)	0.001	15.5	1.9-124.3
B_{38}	4 (8)	17 (34)	0.001	0.2	0.1-0.5
B_{W4}	39 (78)	23 (46)	0.001	4.2	1.7-9.9
C_{W4}	10 (20)	17 (34)	0.120	0.5	0.2-1.2
C_{W7}	7 (14)	19 (38)	0.010	0.3	0.1-0.7

Data are shown as frequency (%), p-value = 0.05 is statistically significant, CI: Confidence interval

The rate of HLA_I positivity in the two studied groups is summarized and compared in Table 1. Accordingly, the rate of A_2-positive cases was significantly higher in the patients than in the controls (58 vs. 32%, p = 0.01, Odds Ratio (OR) = 2.9).

Similar finding was documented in terms of A_3 (62 vs. 26%, p<0.001, OR = 4.6), B35 (24 vs. 2%, p = 0.001, OR = 15.5) and B_{W4} (78 vs. 46%, p = 0.001, OR = 4.2).

In contrast, the rate of B_{38}-positive (34 vs. 8%, p = 0.001, OR = 0.2) and C_{W7}-positive (38 vs. 14%, p = 0.01, OR = 0.3) cases was significantly higher in the hepatitis-C-negative group.

There was no significant difference as for the rate of C_{W4}-positivity between the two studied groups (20% in the cases vs. 34% in the controls, p = 0.12, OR = 0.5).

DISCUSSION

It has been suggested that various HLA alleles might be not only associated with susceptibility of infection

with HCV, but also with its course, severity and extra-hepatic manifestations (Sebastiani *et al*., 2005; Hong *et al*., 2005; Farag *et al*., 2013).

In this study, for the first time, a possible association between various types of HLA-I and HCV infection was investigated in a group of Azeri patients and normal counterparts. Based on the findings, HLA B_{35}, A3, B_{W4} and A_2 alleles were significantly higher in the patients than in the controls (OR = 15.5, 4.6, 4.2 and 2.9, respectively). On the other hand, HLA C_{w7} and B_{37} were against HCV infection (OR = 0.3 and 0.2, respectively). There was no significant difference between the two groups in term of HLA C_{w4}.

A possible association between host genetic diversity and HCV infection has been examined in a number of previous studies. For example, in a study by Tripathy *et al*., 2009) among 43 HCV-positive patients and 67 healthy counterparts, there was a significant association between HLA-I alleles including A_3, A_{32}, B_{15}, B_{55}, C_{w16} and C_{w18} with higher possibility of HCV infection.

Among the studied alleles in this study, only A_3 was also a significant risk factor for HCV infection in our series. This high rate of heterogeneity indicates a strong effect of ethnicity in this regard. In addition, it has been claimed that besides ethnicity, geographical differences also play an important role in this regard (Wang *et al*., 2009; De Almeida *et al*., 2011).

In another study by Paladino *et al*. (2007) in Argentina, it was shown that the presence of HLA-I B_{W4} is a potent risk factor for HCV infection. This allele was also considered as a portent of a worse progression of the infection.

The result of this study is also in conformity with our findings, in which B_{W4} was significantly associated with HCV infection.

In a Tunisian study by Ksiaa *et al*. (2007) on 99 patients with chronic HCV infection (n = 75) or clearance (n = 24), they found out that among HLA-I alleles, B_{35} was more frequently detected in those with viral clearance than in those with chronic infection (21.7 vs. 16.6%).

In our series, as mentioned before, B_{35} was the most potent indicator of HCV infection (OR = 15.5). It is not clear whether this allele may also play a prognostic role in Azeri patients, as well or not. This should be clarified in future studies.

In a series in Pakistan by Anis *et al*. (2007), there was no significant difference between the patients with HCV infection and normal controls in terms of HLA-I alleles.

Again, ethnic and geographical differences may justify the contradicting results of this study comparing with ours.

In a Korean series by Yoon *et al*. (2005), 137 patients with chronic HCV infection were compared with 206 normal counterparts for HLA class I molecules. According to their finding, frequency of A_3 and B_{35} was significantly higher in the patients than in the controls. Surprisingly, both the mentioned alleles were also significantly associated with HCV infection in our series.

In a study in Ireland, McKiernan *et al*. (2004) examined 141 patients with chronic infection and 86 with viral clearance. According to their findings, HLA-I A_3 was associated with clearance (39.5 vs. 19.1%).

The frequency of HLA A_3 allele was 62% in our patients, which is rather higher than both 39.5% and 19.1% in the mentioned study. In addition, this allele was significantly associated with HCV infection in our series. However, the prognostic role of this allele was not evaluated in the present work. This high gap between the rates of expression between the two studies could be attributed to ethnicity, as mentioned earlier.

In another study in Thailand, Vejbaesya *et al*. (2000) did not report a significant difference between A and B alleles of HLA-I between the patients with HCV infection and the healthy group.

This is in contrast with our findings, emphasizing on different ethnicity and geographical area of the patients in two separate studies.

In this brief discussion, the reports of various studies regarding a possible association between HLA and HCV infection are summarized. As underlined earlier, because of a clear heterogeneity among these studies due to ethnic and geographical differences, it is essential that every single ethnicity in every geographical area reports its unique status in this regard. The present work is the first report from Northwestern Iran with Azeri inhabitants in majority. According to this report, different HLA alleles may be considered as a foible point, or in contrast, as a forte for the host against HCV infection. Further studies, especially with regard to possible association between HLA and the course/outcome of infection are recommended.

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