

# Investigation of Human Leukocyte Antigen (HLA) Class I and II Alleles and Hepatitis C Virus (HCV) Genotypes in Patients Infected with HCV

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Hepatitis C virus (HCV) is a major public health problem with more than 130-180 million people infected worldwide. Several studies in different populations have reported the association of human leukocyte antigen (HLA) genotypes, and HCV viral load, and genotypes. The aim of this study was to investigate a possible association between HLA class I and II alleles in HCV-infected patients and healthy individuals. This study was conducted on 82 individuals characterized as group A: 29 healthy screened blood donors as controls; group B: 7 patients positive for HCV antibodies with non-detectable HCV-RNA; and group C: 46 patients positive for HCV antibodies with detectable HCV-RNA. HCV seropositivity was determined by enzyme immuno-assay (EIA), confirmed by recombinant immune blotting assay (RIBA). Viral RNA was detected by qualitative polymerase chain reaction (PCR) followed by determination of viral load by quantitative RT-PCR. HCV DNA amplicons were utilized by immune blotting hybridization assay for detection of HCV genotypes in HCV-infected groups. HLA genotyping was performed for all studied groups. HCV genotype 1 (1a, 1b) was the commonest among the high viral load (> 500.000 copies/ml) HCV-infected patients (70%), while genotype 4 was found in only (18.75%) low viral load (< 500.000 copies/ml) HCV-infected patients. Each group was characterized exclusively by certain HLA genotypes. HLA-A99, B6, B14, B15, B45, B78, DQ3, and DQ22 were only detected in group A subjects. HLA-B70 was observed only in group B, and HLA-A21, A34, A69, B0, B21, B39, B46, B56, B58, B60, B65, B71, B75, DR6, DR9, DR18, and DQ9 were detected in group C only. High frequencies 44.8%, 37.9%, 34.5%, and 27.6% for HLA- DQ6, DQ2, A2 and B51, respectively were observed in healthy controls. Meanwhile, 54.3%, 34.8%, 28.3%, 26.1%, 23.9%, and 17.4% frequencies were observed for HLA-DQ2, DQ6, A1, A2 and DR17, DR4, B51, and B0, respectively in HCV-infected patients. The HLA genetic makeup maybe a contributing factor for determining HCV infection outcome, the virus clearance or chronic persistence infection. Further researches on a larger scale are needed.

**Key words:** HCV infection, viral load, HCV genotypes, HLA genotypes

**H**epatitis C virus (HCV) is a leading cause of viral hepatitis. Acute infection is mild, and asymptomatic in a great majority of patients. The majorities of the infections are persistent, and could finally result in hepatic fibrosis, liver cirrhosis,

hepatocellular carcinoma, and other end-stage liver diseases (1).

Worldwide, 180 million people were reported to have been infected with HCV, and approximately 3-4 million new cases are considered to be infected

by HCV. The prevalence of the disease is  $1 \pm 2\%$  in the United States and Europe, but it can reach 8% in some developing countries (2).

Studies have reported the effect of many factors in disease development such as viral factors including viral genotype and viral load, and host factors including age, sex, environment, associated medical conditions as well as genetic variations (3).

The host immunity can greatly influence the outcomes of HCV infection. The robust and sustained immunological responses mediated by the activation of CD4+, and CD8+ cytotoxic T lymphocytes are the most important factors in determining viral clearance or persistence (4). Both T cells recognize the viral antigens presented on the surface of the infected hepatocytes through binding the T-cell receptors to human leukocyte antigen (HLA) molecules that present the peptide fragments of the viral antigens (4).

Genes encoding HLA molecules (also known as the human major histocompatibility complex), are divided into three sub regions; HLA classes I, II, and III, and are located on chromosome 6p21, a region which encodes many genes which are important for the immune system (5).

HLA cluster is composed of six major genes A, B, C, DR, DQ, and DP. The first three genes of class I influence the presentation of endogenous antigens, while the other three genes which belong to class II influence the presentation of exogenous antigens, affecting therefore the outcome of various infectious diseases (6).

HLA alleles are highly polymorphic among different populations which give variation in immune response. Several studies in different populations demonstrated the association between HLA alleles, and the outcome of HCV infection, but associated alleles are highly variable from one population to another (7).

Although the HLA genotype distribution has been found to differ according to ethnicity, there were no published data concerning this relationship among the Saudi population. Therefore, the aim of

this study was to perform class I, and II HLA-typing in HCV-infected Saudi patients and healthy individuals, determine HCV viral load and genotypes in those patients, and investigate the association between HLA genetic makeup, and the severity and progress of HCV infection.

## **Materials and methods**

### **Patients**

A total of 82 healthy and HCV-infected cases were recruited from Blood Banks and out-patient Clinics of Hepatology and Gastroenterology Department of King Fahed General hospital (KFGH)-MOH, Jeddah, Saudi Arabia. This study was approved by the KFGH-MOH ethical committee.

Cases were divided according to screening analysis of HCV-EIA, and confirmation by RIBA and RT-PCR methods into three groups. Group A contained 29 healthy screened blood donors as a control group, group B was composed of 7 patients' anti-HCV positive / HCV-RNA negative, and group C contained 46 patients' anti-HCV positive / HCV-RNA positive. Serum samples of groups B and C cases were tested by quantitative RT-PCR and genotype assay in order to determine the viral load, and HCV genotypes. The Buffy coat leukocytes of all blood samples were examined by LABType™ SSO kit to identify HLA-type class I and II.

### **Blood collection and sample preparation**

Five ml of venous blood was collected from each subject, and divided into two aliquots. One was collected on plain tubes, centrifuged, and the separated serum was used for standard liver function tests including aminotransferases, alkaline phosphatase, direct and total bilirubin. The other aliquot was collected on anti coagulant dextrose (ACD) tubes for isolation of peripheral blood lymphocytes (PBLs). Buffy coat extraction was performed by Hanks solution. Specimens were stored in the same vacutainer at  $-20^{\circ}\text{C}$  for genomic DNA extraction using commercially available kit (QIAamp DNA Mini and Blood Mini, QIAGEN)

according to manufacturer's instructions.

### HCV detection

For HCV antibodies detection, the collected sera were tested by enzyme immuno-assay (EIA), using commercially available kit (MUREX HCV Ab, ABBOTT Laboratories) according to manufacturer's instructions by using BEP III instrument. Then the results were confirmed by recombinant immune blotting assay (RIBA), using commercially available kit (INNO-LIA HCV Score, Innogenetics) according to manufacturer's instructions.

### HCV viral load and genotyping

Positive sera by both EIA and RIBA were tested by qualitative RT-PCR (COMAS AMPLICOR, HCV Qualitative Test, version 2.0, ROCHE) using primers selected from the highly conserved 5'-UTR of HCV genome. Amplified products were electrophoresed, photographed, and analyzed. Extraction and amplification of RNA were carried out according to the manufacturer's recommendations. Amplicons were genotyped using a commercial reverse phase immune blotting hybridization technique (HCV Genotype Assay LiPA, Bayer Health Care).

### HLA typing

Identification of HLA-type class I and II was performed using a commercially available kit (LABType™ SSO Typing Test, ONE LAMDA) according to manufacturer's instructions, and using Gene Amp PCR System 9700, Applied Biosystem.

### Statistical analysis

Statistical package for social sciences (SPSS/Version 16) software was used. Correlation and association were performed using "t" test, Chi-square (X<sup>2</sup>), and Z test, 95% confidence interval (95% CI), and probability value ( $P \leq 0.05$ ) was considered statistically significant.

## Results

HLA class I, and class II genotypes frequencies and distribution in healthy control (group A) demonstrated that the most frequent

alleles were DQ6 31%, DQ2 29.3%, DR13 25%, B51 24%, A2 20%, and DR11 8% (Table 1). While in HCV-infected patients (group B) the frequency and distribution of HLA class I and class II alleles were DQ2 50%, A2 42.8%, DR7 and DR4 21% each, B50 21.4%, DQ5, DQ6 and DQ7 14% each, B51 and B53 14% each, and A1 14% (Table 2). Regarding the distribution of HCV genotypes among HCV-infected patients (group C) with high viral load (> 500.000 copies/ml), it was demonstrated that HCV genotype 1 was the commonest one (70%), followed by genotype 4 (23.3%), while genotype 3 was found in only 6.6% of infected patients. Frequencies of HLA class I and class II genotypes among this group were DQ2 28%, DQ6 23%, A2 20.8%, DR13 and DR4 16% each, and B50 and B51 11% each (Table 3). Moreover, among HCV-infected patients (group C) with low viral load (< 500.000 copies/ml), HCV genotype 1 was (81.25%), while genotype 4 was (18.75%). Frequencies of HLA class I and class II genotypes among this group were DQ2 and DQ6 37.5% each, DR17 37.5%, B51 37.5%, and A1 and A2 25% each (Table 4).

Among the studied groups, each one was characterized exclusively by certain HLA genotypes; HLA-A99, B6, B14, B15, B45, B78, DQ3, and DQ22 were detected in group A, HLA-B70 in group B, and HLA-A21, A34, A69, B0, B21, B39, B46, B56, B58, B60, B65, B71, B75, DR6, DR9, DR18, and DQ9 were detected in group C, as shown in Figures 1- 4.

High frequencies of 44.8%, 37.9%, 34.5% and 27.6% for HLA- DQ6, DQ2, A2, and B51 were observed in healthy controls, respectively. Meanwhile, 54.3%, 34.8%, 28.3%, 26.1%, 23.9%, and 17.4% for HLA-DQ2, DQ6, A1, A2 and DR17, DR4, B51, and B0 were observed in HCV-infected patients, respectively (Tables 5 and 6).

Statistically significant differences for HLA-A, B, and DQ genotypes were detected when comparing different studied groups (Table 7).

**Table 1.** HLA class I and class II typing for healthy controls (group A)

No	Sex	DR		DQ		B locus		A locus	
1	M	DR4	DR13	DQ6	DQ6	B7	B51	A1	A68
2	M	DR13	DR13	DQ6	DQ6	B8	B51	A3	A68
3	M	DR11	DR16	DQ7	DQ5	B18	B51	A24	A26
4	M	DR11	DR13	DQ3	DQ6	B51	B51	A3	A68
5	F	DR3	DR11	DQ2	DQ6	B42	B45	A30	A30
6	M	DR1	DR7	DQ2	DQ5	B72	B50	A2	A3
7	M	DR7	DR13	DQ6	DQ22	B49	B50	A2	A23
8	M	DR3	DR7	DQ2	DQ4	B7	B8	A24	A29
9	M	DR1	DR3	DQ2	DQ2	B6	B14	A30	A33
10	M	DR13	DR13	DQ6	DQ2	B7	B49	A2	A68
11	M	DR3	DR13	DQ3	DQ5	B41	B42	A30	A66
12	F	DR7	DR7	DQ2	DQ3	B41	B50	A2	A23
13	F	DR3	DR4	DQ2	DQ6	B8	B53	A1	A1
14	F	DR10	DR11	DQ3	DQ6	B49	B57	A1	A2
15	F	DR3	DR4	DQ2	DQ5	B7	B8	A24	A29
16	M	DR4	DR15	DQ6	DQ6	B51	B78	A31	A31
17	F	DR13	DR13	DQ3	DQ2	B35	B52	A1	A24
18	F	DR8	DR15	DQ5	DQ5	B51	B51	A24	A31
19	M	DR11	DR13	DQ3	DQ6	B38	B49	A2	A23
20	M	DR7	DR15	DQ2	DQ2	B50	B51	A2	A33
21	M	DR1	DR7	DQ3	DQ5	B51	B73	A2	A2
22	F	DR11	DR11	DQ3	DQ3	B8	B8	A11	A11
23	M	DR3	DR3	DQ2	DQ2	B6	B42	A1	A2
24	M	DR3	DR3	DQ2	DQ2	B51	B58	A2	A33
25	M	DR11	DR15	DQ3	DQ3	B7	B55	A2	A26
26	F	DR13	DR14	DQ5	DQ5	B51	B51	A24	A26
27	F	DR1	DR3	DQ2	DQ2	B52	B52	A2	A3
28	F	DR13	DR15	DQ6	DQ6	B15	B15	A24	A80
29	F	DR13	DR13	DQ6	DQ6	B51	B51	A1	A23

**Table 2.** HLA class I and class II typing for healthy controls (group A)

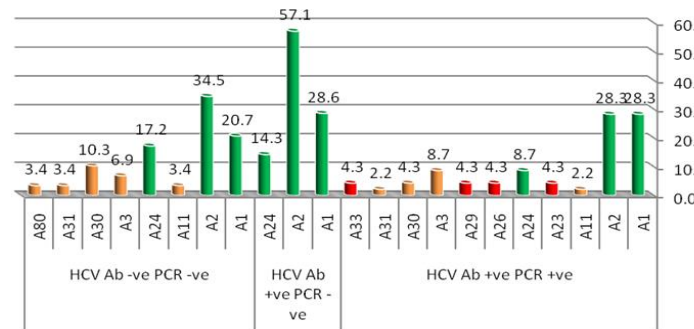
No	Sex	DR		DQ		B locus		A locus	
1	M	DR7	DR17	DQ2	DQ2	B50	B61	A2	A32
2	F	DR1	DR4	DQ2	DQ5	B44	B53	A2	A30
3	F	DR4	DR11	DQ2	DQ7	B18	B57	A24	A32
4	F	DR4	DR15	DQ6	DQ8	B51	B51	A2	A3
5	M	DR7	DR10	DQ2	DQ5	B50	B53	A2	A2
6	F	DR7	DR11	DQ2	DQ7	B50	B70	A1	A2
7	M	DR13	DR17	DQ2	DQ6	B 8	B41	A1	A3

**Table 3.** Distribution of HCV genotypes among group C patients with high viral load, and HLA class I, and class II typing

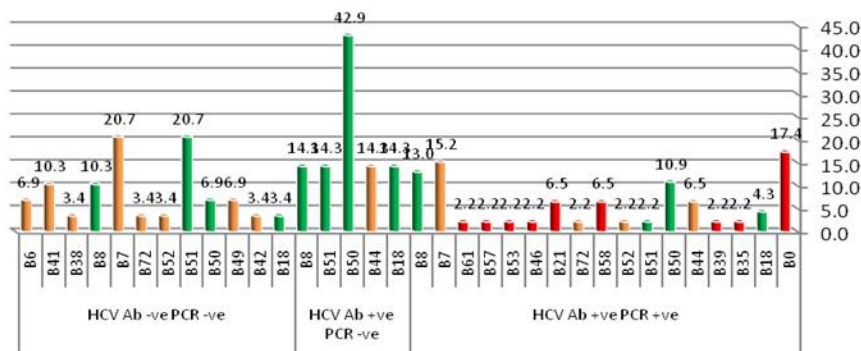
N	Viral copy numbers	Genotype	DR		DQ		B locus		A locus	
1	541,000	4	DR4	DR4	DQ8	DQ2	B72	B52	A24	A2
2	723,000	4	DR13	DR4	DQ8	DQ6	B51	B0	A32	A26
3	627,000	4	DR15	DR13	DQ7	DQ5	B75	B18	A80	A33
4	>500,000	1	DR13	DR11	DQ6	DQ5	B55	B50	A69	A24
5	>500,000	4	DR15	DR15	DQ6	DQ6	B51	B7	A2	A1
6	>500,000	1	DR15	DR4	DQ6	DQ4	B51	B0	A11	A1
7	>234,000	1	DR13	DR10	DQ7	DQ5	B44	B13	A69	A30
8	>500,000	1	DR10	DR1	DQ5	DQ5	B73	B7	A29	A2
9	>500,000	1	DR17	DR7	DQ2	DQ2	B71	B53	A30	A3
10	>500,000	1	DR13	DR6	DQ6	DQ4	B58	B7	A29	A1
11	>500,000	4	DR17	DR8	DQ8	DQ2	B58	B50	A33	A33
12	>500,000	4	DR11	DR4	DQ8	DQ5	B51	B0	A2	A1
13	>500,000	4	DR13	DR13	DQ6	DQ6	B78	B7	A68	A24
14	>500,000	1	DR17	DR11	DQ7	DQ2	B39	B8	A26	A1
15	>500,000	3	DR15	DR4	DQ8	DQ6	B52	B0	A26	A1
16	>500,000	1	DR11	DR4	DQ8	DQ7	B49	B44	A2	A1
17	622,000	1	DR17	DR4	DQ8	DQ2	B60	B13	A68	A30
18	>500,000	1	DR10	DR4	DQ5	DQ2	B50	B44	A2	A2
19	627,000	1	DR10	DR7	DQ5	DQ2	B50	B7	A29	A2
20	700,000	3	DR14	DR1	DQ5	DQ5	B49	B46	A26	A2
21	4,200,000	1	DR16	DR14	DQ5	DQ5	B0	B0	A24	A2
22	650,000	1	DR17	DR7	DQ2	DQ2	B50	B0	A68	A2
23	1,060,000	1	DR7	DR4	DQ2	DQ2	B39	B72	A3	A2
24	573,000	1	DR17	DR7	DQ2	DQ2	B50	B8	A31	A24
25	1,920,000	1	DR15	DR4	DQ6	DQ2	B18	B8	A23	A23
26	4,630,000	1	DR17	DR7	DQ2	DQ2	B58	B50	A32	A2
27	1,300,000	1	DR7	DR1	DQ5	DQ2	B65	B35	A33	A3
28	1,580,000	1	DR15	DR7	DQ6	DQ2	B50	B18	A31	A23
29	642,000	1	DR7	DR7	DQ2	DQ2	B35	B13	A21	A11
30	2,140,000	1	DR13	DR1	DQ6	DQ5	B51	B58	A33	A2

**Table 4.** Distribution of HCV genotypes among group C patients with low viral load, and HLA class I and class II typing

N	Viral copy numbers	Genotype	DR	DQ	B locus	A locus
1	127,000	4	DR7 DR4	DQ8 DQ2	B44 B44	A24 A3
2	159	1	DR15 DR11	DQ7 DQ6	B51 B57	A2 A2
3	46,100	1	DR17 DR1	DQ5 DQ2	B51 B50	A23 A2
4	319,000	4	DR13 DR1	DQ6 DQ5	B0 B0	A11 A2
5	<600	1	DR17 DR9	DQ9 DQ2	B51 B58	A33 A1
6	56,500	1	DR13 DR7	DQ6 DQ2	B51 B51	A31 A31
7	44,300	1	DR17 DR13	DQ6 DQ2	B51 B8	A31 A1
8	436,000	1	DR14 DR10	DQ5 DQ5	B75 B61	A11 A1
9	232,000	1	DR13 DR3	DQ6 DQ2	B44 B8	A34 A26
10	38,500	1	DR17 DR4	DQ8 DQ2	B50 B8	A32 A3
11	381,000	1	DR15 DR13	DQ6 DQ6	B51 B7	A31 A29
12	3,070	4	DR15 DR15	DQ6 DQ6	B35 B7	A2 A1
13	432,000	1	DR14 DR7	DQ5 DQ2	B55 B50	A24 A1
14	73,800	1	DR17 DR16	DQ5 DQ2	B35 B0	A24 A24
15	36,000	1	DR17 DR13	DQ6 DQ2	B56 B58	A33 A1
16	130,000	1	DR18 DR13	DQ6 DQ4	B42 B39	A32 A29



**Figure 1.** Distribution of HLA class IA among different studied groups.



**Figure 2.** Distribution of HLA class IB among different studied groups.

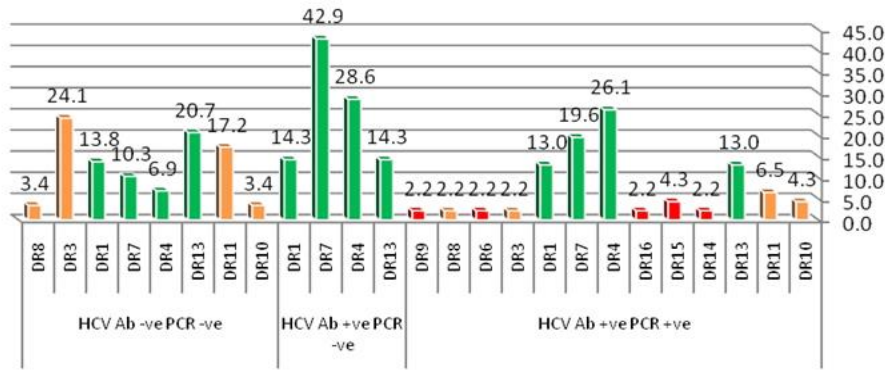


Figure 3. Distribution of HLA class II DR among different studied groups.

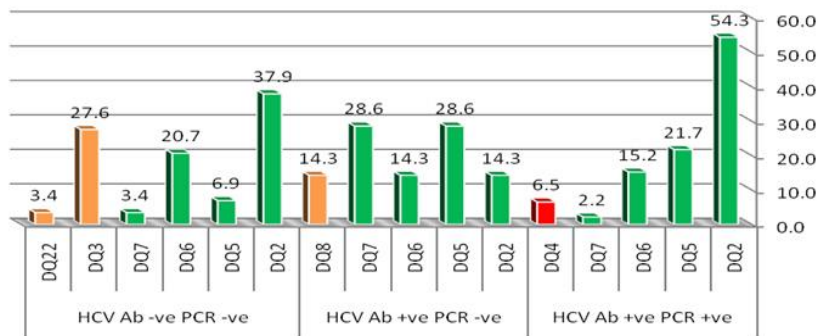


Figure 4. Distribution of HLA class II DQ among different studied groups.

Table 5. Frequencies of HLA genotypes among healthy controls (group A)

Group A	Type	%	(95% CI)
A locus	A2	34.5	20.7 - 48.2
B locus	B51	27.6	14.7 - 40.5
DQ	DQ2	37.9	23.9 - 52.0
	DQ6	44.8	30.5 - 59.2

Table 6. Frequencies of HLA genotypes among HCV-infected patients (group C)

Group C	Type	%	(95% CI)
Quantitative test	High	84.8	74.4 - 95.2
Genotype	Type 1	78.3	66.3 - 90.2
A locus	A1	28.3	15.2 - 41.3
	A2	28.3	15.2 - 41.3
B locus	B0	17.4	6.4 - 28.3
	B51	23.9	11.6 - 36.2
DR	DR4	26.1	13.4 - 38.8
	DR17	28.3	15.2 - 41.3
DQ	DQ2	54.3	40.0 - 68.7
	DQ6	34.8	21.0 - 48.5

Table 7. Statistical comparison of results among the studied groups

Group	A		B		C	
	P-value	X2	P-value	X2	P-value	X2
Quantitative	-	-	-	-	<b>0.000*</b>	22.26
Genotype	-	-	-	-	<b>0.000*</b>	42.96
A locus	<b>0.006*</b>	19.83	0.368	2.00	<b>0.000*</b>	47.74
	0.894	6.41	0.934	0.43	0.436	15.22
B locus	0.170	15.28	0.683	2.29	<b>0.007*</b>	33.09
	<b>0.023*</b>	26.34	0.982	0.71	<b>0.000*</b>	47.04
DR	0.195	9.90	0.666	1.57	<b>0.000*</b>	44.43
	0.067	13.21	0.931	0.86	<b>0.000*</b>	34.87
DQ	<b>0.003*</b>	17.97	0.931	0.86	<b>0.000*</b>	39.22
	<b>0.004*</b>	15.66	0.059	3.57	<b>0.003*</b>	17.91

\*Significant ( $P \leq 0.05$ ).

## Discussion

Several studies in different populations demonstrated the association between HLA alleles, and the outcome of HCV infection, but associated alleles are highly variable from one population to another (7). Whereas, the HLA genotype distribution has been found to differ according to ethnicity, there were no published data concerning this relationship among the Saudi population.

HLA class I and class II genotypes analysis in healthy controls demonstrated that the most frequent alleles were DQ6 31%, DQ2 29.3%, DR13 25%, B51 24%, A2 20%, and DR11 8%. There was a statistically significant difference for HLA-A, B and DQ genotypes among healthy normal Saudi populations. The findings of the present study on HCV infected patients are in accordance with a number of studies having reported a possible association between HLA genotype and HCV infection. In a study conducted by Chowdhry et al. A\*02, and A\*31 alleles frequency decreased significantly in anti-HCV-positive patients (8). Frequencies for HLA-B loci did not reach any statistical significance. Among the class II alleles, HLA-DRB1\*03, and HLA-DRB1\*10 were

significantly higher in the patients population, and HLA-DRB1\*15 was significantly decreased in the patients population in comparison with the controls. Moreover, Xiong et al. reported that the frequencies of four HLA alleles, B\* 07:05, B\*13:02, B\*15:01, and B\*15:02, were significantly different between the HCV-infected, and uninfected blood donors in China, revealing an inverse relation between B\*15:01 and B\*15:02, and HCV infection (4). This finding suggests that the ethnic genetic variations of HLA may greatly affect the host immune response against HCV.

Also, Gheorghe et al. showed that the HLA-DRB1\*0301 allele had a high frequency (14.8%) in null-responders while DRB1\*0701 (11.1%), DRB1\*11 (22.2%), and DRB1\*0101 (16.7%) alleles were prevalent in sustained virologic responders. No significant correlation was found between the presence of HLA-DRB1\* alleles, and viral loads or liver fibrosis in this group of Romanian patients (9).

Comparing the distribution of HLA-DRB1 and -DQB1 alleles between HCV patients, and healthy group by Shaker et al. revealed that DQB1\*0205, DQB1\*0303, DQB1\*0312, DQB1\*0315, DQB1



\*02, DQB1\*03, DQB1\*02, DQB1\*03 and DRB1\*07 alleles were more frequent in healthy controls than in patients. Those alleles may enhance the immune response against HCV infection. However, DQB1\*0201, DQB1\*0202, DQB1\*0204, DQB1\*0301, DQB1\*0309, DQB1\*0319, DQB1\*06, and DRB1\*13 alleles were significantly more frequent in Egyptian patients than in healthy group (7).

In another study performed in Iraq, the presence of HLA-DR5 (odd ratio 2.7,  $P=0.022$ ) and HLA-DQ2 (odd ratio 3.1,  $P=0.008$ ), and the absence of HLA-DR7 (inverse odd ratio 3.2,  $P=0.04$ ) and HLA-DQ1 (inverse odd ratio 2.8,  $P=0.012$ ) were associated with significantly increased risk for HCV infection as compared to healthy controls (10). On the other hand, the presence of HLA-DR5 (odd ratio 6.5,  $P=0.005$ ) and HLA-DQ2 (odd ratio 5.1,  $P=0.002$ ), and the absence of HLA-DR7 (inverse odd ratio 4.8,  $P=0.018$ ) were significantly associated with increased risk for HCV infection.

Furthermore, Ocal et al. found a possible association between the course of HCV infection, and specific HLA alleles. HLA class I Cw\*6 and HLA class II DRB\*10 alleles were observed more frequently in the viral clearance group ( $P<0.05$ ). The HLA class I B\*38 allele group was more prone to develop chronic hepatitis C ( $P<0.01$ ) (1).

Also, HLA-DRB1\*11, DRB3 and DQB1\*03 had significantly higher association with subjects with self limiting disease, and sustained viral response to treatment among Egyptian population (3). While the HLA-DRB4 showed higher association with non responders to therapy. So, these results confirm that HLA class II type might affect the outcome of HCV infection, and response to treatment. Furthermore, Cho et al. showed that multiple HLA class II DRB1\*1302, DRB1\*1502, DQB1\*0302, and DQB1\*0609 alleles, and related-haplotypes were associated with viral clearance, while DRB1\*0701 and DQB1\*0301 alleles, and related-haplotypes were associated with persistence,

in HBV-infected Korean individuals (11).

In the present study, the most prevalent genotype among HCV-infected patients was found to be genotype 1 (1a, 1b) with a prevalence rate of (81.25%), and the second prevalent genotype was genotype 4 with a prevalence rate of (18.75%) which is in line with the previous studies. In agreement with our findings, a study conducted by Haefelin et al. indicated that the protective effect of HLA-B27 is limited to HCV genotype 1 infection, and does not expand to other genotypes such as genotype 3a (12). These results underline the central role of a single HLA-B27-restricted epitope-specific CD8<sup>+</sup> T-cell response in mediating protection in HCV genotype 1 infection in German population.

Another study performed in the Persian Gulf region, revealed that the prevalence of various HLA antigens differed significantly between various countries, including Saudi Arabia. The investigators also reported that HLA-DR2, which has the highest prevalence worldwide, had varying prevalence rates in the studied Persian Gulf countries, with the Omani population having the highest prevalence of HLA-DR2 (13). Correspondingly, HLA-A, -B, -C and DR loci antigen frequencies were determined by Sheth et al. on normal Saudi subjects. And it was found that B21, CW4, CW7, and DR7 had the highest frequencies, which is comparable to the results of the present study.

A study conducted by Fitzmaurice et al. reported a potential protective impact of HLA-A on HCV infection (14). Relatively, HLA-DRB1\*03 individually ( $P=0.025$ ) or in combination with HLA-DRB1\*04 ( $P=0.035$ ) were found to be significant protective alleles against HCV infection (15). Among Egyptian patients on interferon therapy, HLA-DRB1\*11 was significantly associated with viral clearance. In contrast, HLA-DRB1\*07 ( $P=0.005$ ) was associated with viral persistence (15).

The HLA-DRB1\*07 allele was found to be significantly associated with virus persistence, whilst HLA-DQB1\*0301 was found to be associated

with virus clearance in a study in Pakistan (16). In the non-sustained viral response group, HLA-DRB1\*07 was found as the most common allele (27.8%), whereas it was found at lower levels in the uninfected control group (16.7%), and also in the sustained viral response group (13.0%). HLA-DRB1\*11 was found in the control and sustained viral response groups at 15.2 and 14%, respectively, and was found at lower levels in the non-sustained viral response group (5.6%). HLA-DRB1\*04 was found at a relatively higher level in the control group (11.8%) compared with the other groups. The DQB1\*0301 allele was found at similar frequencies in the control (16.7%), and sustained viral response (17.7%) groups, whereas in the non-sustained viral response group it was found at only 6.5%.

Another analysis revealed an association of HLA alleles HLA A\*03 (OR= 16.69, EF 0.44, P= 7.9E-12), A\*32 (OR= 1474, EF 0.21, P= 1.8E-9), HLA B\*15 (OR=14.11, EF 0.39, P= 2.18E-10), B\*55 (OR= 12.09, EF 0.07, P= 0.005), Cw\*16 (OR= 7.45, EF 0.12, P= 0.001), Cw\*18 (OR= 402, EF 0.05, P= 0.003), DRB1\*03 (OR= 4.01, EF 0.08, P= 0.01), and DQB1\*03 (OR= 3.02, EF 0.22, P= 0.001), with HCV infection. HLA II locus haplotype [DRB1\*11;DQB1\*03] (HF 17.64, OR=5.16, P= 0.0001) frequency was significantly increased among HCV infected Indian population (17). On the other hand, Al-husain et al. reported that no association was found between HLA genotypes, and HCV infection in Saudi haemodialysis patients with HCV infection (18).

From the current study we concluded that HLA genetic makeup maybe a contributing factor for determining HCV infection outcome, the virus clearance or chronic persistence infection. Accurate testing of blood donors, and high risk individuals for prevention of HCV infection are recommended. Researches on larger sample sizes are needed to further elucidate the association between HLA alleles and predisposition to HCV infection and/or clearance.

#### Conflict of interest

The authors declare that they have no competing interest.

#### References

1. Ocal S, Selcuk H, Korkmaz M, et al. Effect of HLA on hepatitis C virus clearance and persistence in anti-HCV-positive end-stage renal disease patients. *Saudi J Gastroenterol.* 2014;20:175-81.
2. Ruiz-Extremera A, Pavon-Castillero E J, Florido M, et al. Influence of HLA class I, HLA class II and KIRs on vertical transmission and chronicity of hepatitis C virus in children. *PLoS One.* 2017;12:e0172527.
3. Raouf M M E M, Ghoneim F M, Aly H A S, et al. HLA Class II Genotyping in Chronic HCV Patients. *Egypt J Med Microbiol.* 2017;26:77-82.
4. Xiong H, Huang J, Rong X, et al. HLA-B alleles B\*15:01 and B\*15:02: opposite association with hepatitis C virus infection in Chinese voluntary blood donors. *Intervirology.* 2015;58:80-7.
5. Shahin a A, Shaker O G, Darweesh H E, et al. Does human leukocyte antigen influence the risk of development and type of vasculitis in Egyptian patients with chronic hepatitis C virus infection? *Egypt Rheumatol.* 2016;38:307-12.
6. Yue M, Xu K, Wu M P, et al. Human Leukocyte Antigen Class II Alleles Are Associated with Hepatitis C Virus Natural Susceptibility in the Chinese Population. *Int J Mol Sci.* 2015;16:16792-805.
7. Shaker O, Bassiony H, El Raziky M, et al. Human leukocyte antigen class II alleles (DQB1 and DRB1) as predictors for response to interferon therapy in HCV genotype 4. *Mediators Inflamm.* 2013;2013: 392764
8. Chowdhry M, Makroo R, Singh M, et al. Human leucocyte antigen Class I and II alleles associated with anti-hepatitis C virus-positive patients of North India. *Indian J Med Microbiol.* 2016;34:299-302.
9. Gheorghe L, Rugina S, Dumitru I M, et al. HLA class II alleles in Romanian patients with chronic hepatitis C. *Germes.* 2015;5:44-9.
10. Hasan A-R S and Al-Ajeeli K S A. Association of Hepatitis C Virus (HCV) Infection and Human Leukocyte Antigens (HLA). *Diyala Journal of Medicine.* 2012;2:53-9.
11. Cho S W, Cheong J Y, Ju Y S, et al. Human leukocyte antigen class II association with spontaneous recovery from hepatitis B virus infection in Koreans: analysis at the haplotype level. *J Korean Med Sci.* 2008;23:838-44.

12. Neumann-Haefelin C, Timm J, Schmidt J, et al. Protective effect of human leukocyte antigen B27 in hepatitis C virus infection requires the presence of a genotype-specific immunodominant CD8+ T-cell epitope. *Hepatology*. 2010;51:54-62.
13. White a G, Leheny W, Kuchipudi P, et al. Histocompatibility antigens in Omanis: Comparison with other Gulf populations and implications for disease association. *Ann Saudi Med*. 1999;19:193-6.
14. Fitzmaurice K, Petrovic D, Ramamurthy N, et al. Molecular footprints reveal the impact of the protective HLA-A\*03 allele in hepatitis C virus infection. *Gut*. 2011;60:1563-71.
15. Shaala A, Harfoush R, Elkhoully E, et al. Could Human Leukocyte Antigens (HLA) be Predictive Factors to Interferon Response among Chronic Hepatitis C Virus Hepatitis. *J Medical Microbiol Diagnosis S*. 2012;1:2161-0703.
16. Ali L, Mansoor A, Ahmad N, et al. Patient HLA-DRB1\* and -DQB1\* allele and haplotype association with hepatitis C virus persistence and clearance. *J Gen Virol*. 2010;91:1931-8.
17. Tripathy a S, Shankarkumar U, Chadha M S, et al. Association of HLA alleles with hepatitis C infection in Maharashtra, western India. *Indian J Med Res*. 2009;130:550-5.
18. Alzahrani a J, Obeid O E, Alali a K, et al. Assessment of Genetic and Immunological Factors Associated with Hepatitis C Infection in Saudi Hemodialysis Patients. *J Biosci Med*. 2014;2:51-7.