ORIGINAL ARTICLE

# HLA DRB1 Alleles in Association with Chronic Hepatitis C Virus Infection and Autoimmune Hepatitis

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***Key words:***
***HCV;Autoimmune hepatitis; HLA typing; HLA DRB; autoantibodies***

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## ABSTRACT

***Background:*** *Hepatitis C virus is a hepatotropic and lymphotropic virus that has been associated with various diseases and syndromes. Autoimmune hepatitis is a chronic disease of unknown etiology, marked by the continuous inflammation of hepatocytes and necrosis and has a tendency to advance to cirrhosis.* ***Objective:*** *A comparative study between cases of chronic HCV infection and AIH cases regarding different parameters including their genetic association with HLA class II alleles: DRB1*01* - DRB1*15**
***Methodology:*** *The study included Eighty subjects, Group I: 30 chronic HCV, Group II: 30 AIH, Group III: 20 healthy controls. HLA Typing was performed using a line probe assay.* ***Results:*** *HLA DRB1*07, DRB1*11 were highly associated with HCV clinical cases. DRB1*3 and DRB1*5 were the predominant alleles in AIH (representing 66.7% and 53.3% respectively).* ***Conclusion:*** *Certain HLA alleles of class II could be linked to hepatitis cases (either caused by HCV or autoimmune) and can predict clinical course and outcome of the disease.*

## INTRODUCTION

The liver is considered the largest organ in an adult body [1,2]. Multiple causes of liver inflammation including; infections, malnutrition, intoxication, metabolic hereditary disorders and immune mediated diseases, can result in liver damage and eventually failure [3].

Hepatitis C virus (HCV) infection is a significant cause of chronic liver disease worldwide, and is accompanied with high morbidity and mortality. Globally, WHO estimates that there were 71 million people living with chronic HCV infection in 2016, and 399,000 deaths in 2015, mainly from cirrhosis or hepatocellular carcinoma (HCC)[4]. In addition, there were approximately 1.75 million new infections per year [4,5].

In 2016, the WHO global health sector strategy on viral hepatitis outlined global targets and priority actions for countries to achieve the goal of eliminating viral hepatitis which is considered a public health threat by year 2030. Elimination was defined as a reduction in mortality by 65% and in the incidence of chronic infections by 90% [6].

In contrast to other flavivirus infections, the majority of adults with primary HCV infection develop persistent infection. Persistence must therefore relate to, either the inability of the host to mount an effective immune response or viral factors that facilitate immune evasion, or perhaps to combination of both factors. In addition, although HCV is primarily a hepatotrophic virus it either causes or is related to several other diseases both hepatic and non-hepatic [7].

Chronic hepatitis is also caused by diverse non-viral aetiologies, diseases such as Wilson's disease, alpha-1 anti-trypsin deficiency and autoimmune hepatitis (AIH) are uncommon, while alcohol-associated liver injury and hepatitis associated with medications and herbal remedies are frequently seen in liver clinics. Autoimmune hepatitis (AIH) is a relatively rare disease[8], belonging to the group of the autoimmune liver diseases (AILD) [9]. AIH is a chronic hepatitis, marked by self-perpetuating and progressive inflammation of liver structures, due to aberrant autoreactivity [8-10]. Ultimately, this disease can advance to cirrhosis and subsequently to liver failure and HCC. Consequently, AIH is an important cause of morbidity and mortality related to liver disease[11].

Prevalence of AIH ranges from 10 to 17 per 100.000 in the Caucasian people. Some studies mention a prevalence of 24.5 and 42.9 cases per 100000 in respectively New Zealand and Alaska. Thus, the prevalence in our area may be underestimated. AIH has a worldwide distribution and can occur at any age, in both genders and in every ethnic group[12].

The pathogenesis of AIH is still unknown, although both environmental and genetic factors are thought to be involved in the initiation of this AILD. The most accepted theory of the mechanism inducing AIH, postulates that an environmental agent triggers the immune system of a genetic susceptible patient [13]. Several observations confirm an inherited liability for

AIH. AIH does not adhere to a Mendelian mode of inheritance, meaning that no single gene has been identified as responsible for this illness. The entire genetic base is still not elucidated [14].

Studies by Teufel et al [15] and Baharlou et al [16] demonstrated a distinct genetic association of the human leukocyte antigen (HLA) region with AIH. Located on chromosome 6p21.3, the strongest association can be found in the major histocompatibility complex (MHC) class II region (HLA-DR) [14]. Various HLA serotypes are linked to different ethnicities and races [17,18]. The two main alleles, HLA- DRB1*03 or DRB1*04 confer a 6-7 fold increased disease risk. Though it plays the dominant role, the HLA-region alone cannot explain the whole genetic base of AIH. In addition, a genome-wide association study (GWAS) has identified several genetic loci outside the region of HLA considering to take part in the disease mechanism [16].

The current work is a comparative study among cases of HCV infection and AIH regarding CBC, liver functions and presence of autoantibodies. Also, to find out the genetic association among (HLA class II alleles: DRB1*01* - DRB1*15*) and HCV and AIH clinical cases.

## METHODOLOGY

**Subjects:**

This study was carried on Sixty adult patients (aged from 15-65 years, 27 males and 33 females) with abnormal liver function tests (30 chronic HCV and 30 autoimmune hepatitis free of HBV and HCV infection) selected from Alexandria Armed Forces Hospital, in addition to 20 age and sex matched healthy controls. An informed consent was obtained from all enrollees before sampling. The university ethical committee approved the study design in May 2017 (IRB:00007555 - FWA:00015712), Serial number:0105043.

**Methods:**

Full history was obtained from all patients, and they were clinically examined. All the recruited subjects were put through the following tests:

- **Complete blood count;** done by an automated cell counter (Roche, Japan).
- **Liver function tests** [including; Alanine transaminase (ALT), Aspartate transaminase (AST), bilirubin, albumin, alkaline phosphatase, gamma glutamyl transferase (GGT) and Prothrombin time (PT)].
- **Detection of HBsAg**, HBeAg, HBc IgM by ELISA (Behring), and HCV Ab using 3rd generation ELISA technique.
- **Real time polymerase chain reaction (RT- PCR)** for detection and quantitation of HCV RNA.
- **Detection and semiquantitation of; anti-nuclear antibodies** (ANA), anti-smooth muscle antibody (ASMA), anti-mitochondrial antibody (AMA), anti-liver/kidney microsomal antibody (AL/KM), and perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) auto-antibodies using ImmcoTM auto-antibody test system by indirect immunofluorescent technique (IF), while anticentromere antibodies (ACA) was performed by commercial enzyme linked immunosorbent assay (ELISA). For (ANA, ASMA, and AMA), sera of patients were added to mouse kidney/stomach/liver substrate sections and for p-ANCA, sera were added to human neutrophil substrate, for the binding of specific antibodies to substrate. Unbound antibody was eliminated by washing the slide. Then a substrate with fluorescein labelled-antihuman IgG conjugate was added to aid the detection of bound antibodies of IgG class. The slides were examined by the fluorescent microscopy, the presence of autoantibodies was demonstrated by an apple green fluorescence. Serial dilutions were tested to estimate the accurate titer, a titer of <20 was reported as a negative result.
- **HLA class II (DRB1*01* - DRB1*15*) typing:** Using; a line probe assay designed for molecular typing of HLA alleles at the allele group level (One typing strip have 37 sequence- specific DNA probes and 2 control probes fixed on it). Genomic DNA was extracted from blood (after lysis by lysing buffer) using Qiagen spin column QIA amp® DNA Blood Mini Kit. Alleles of HLA classII were detected at the genotype level with 2digit intermediate/low resolution. This was performed using INNO-LiPA plus; a line probe assay designed for molecular typing of HLA alleles at the allele group level. The principle of the test was based on the reverse hybridization of the amplified biotinylated DNA sample which was chemically denatured, and the separated strands were hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. A stringent wash step followed to detach any ill-matched amplified DNA. Then, adding streptavidin conjugated to alkaline phosphatase in order to bind any previously formed biotinylated hybrid. Finally, a chromogenic substrate was added resulting in a purple/brown precipitate, and the reactivity pattern of the probe was recorded.

**Statistical analysis:**

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).

## RESULTS

As regards sex, there was a significant male predominance among chronic HCV clinical cases (80%) while female predominance was detected among AIH cases (90%). These findings were statistically significant ($p \leq 0.001$*) (table 1).

**Table 1: Comparison between the three studied groups according to demographic data**

| | Chronic HCV (n=30) | | Autoimmune (n=30) | | Control (n=20) | | Test of Sig. | p |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | | |
| **Sex** | | | | | | | | |
| Male | 24[a] | 80.0 | 3[b] | 10.0 | 12[a] | 60.0 | $\chi^2$= | <0.001* |
| Female | 6[a] | 20.0 | 27[b] | 90.0 | 8[a] | 40.0 | 30.769* | |
| **Age** | | | | | | | | |
| Min. – Max. | 24.0 – 59.0 | | 28.0 – 58.0 | | 19.0 – 40.0 | | F= | |
| Mean ± SD. | 46.77 ± 8.95 | | 44.50 ± 9.22 | | 29.65 ± 4.88 | | 28.713* | <0.001* |
| Median (IQR) | 48.0(42.0 –55.0) | | 43.50(38.0 –55.0) | | 29.50(28.0 –32.0) | | | |
| **Sig. bet. grps.** | $p_1$=0.539, $p_2$<0.001*, $p_3$<0.001* | | | | | | | |

$\chi^2$: Chi square test

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey) p: p value for comparing between the studied groups

$p_1$: p value for comparing between Chronic HCV and Autoimmmune

$p_2$: p value for comparing between Chronic HCV and Control

$p_3$: p value for comparing between Autoimmmune and Control

*: Statistically significant at $p \leq 0.05$

Common letters are not significant (i.e. Different letters are significant)

The laboratory findings of blood picture showed that the two studied groups of patients demonstrated a significant decrease in platelet count and in haemoglobin compared to controls ($p \leq 0.001$*), while the Hb level was significantly higher in the chronic HCV clinical cases compared to AIH cases (p = 0.001*) (table 2).

**Table 2: Comparison between the three studied groups according to CBC**

| | Chronic HCV (n=30) | Autoimmune (n=30) | Control (n=20) | F | p |
|---|---|---|---|---|---|
| **Platelets** | | | | | |
| Min. – Max. | 110.0 – 310.0 | 60. 0 – 330.0 | 220.0 – 430.0 | 45.885* | <0.001* |
| Mean ± SD. | 197.8 ± 42.89 | 169.8 ± 71.25 | 324.0 ± 54.59 | | |
| Median (IQR) | 195.0(175.0 –210.0) | 175.0(100.0 –210.0) | 310.0(290.0 –370.0) | | |
| **Sig. bet. grps.** | $p_1$=0.154, $p_2$<0.001*, $p_3$<0.001* | | | | |
| **Hb (g/dl)** | | | | | |
| Min. – Max. | 9.50 – 15.0 | 5.0 – 14.0 | 11.70 – 17.0 | 34.825* | <0.001* |
| Mean ± SD. | 11.65 ± 1.44 | 9.91 ± 2.33 | 14.37 ± 1.56 | | |
| Median (IQR) | 11.35(10.60 –13.0) | 9.90(8.70 –12.0) | 14.25(13.10–15.75) | | |
| **Sig. bet. grps.** | $p_1$=0.001*, $p_2$<0.001*, $p_3$<0.001* | | | | |

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey) p: p value for comparing between the studied groups

p1: p value for comparing between Chronic HCV and Autoimmmune

p2: p value for comparing between Chronic HCV and Control

p3: p value for comparing between Autoimmmune and Control

*: Statistically significant at $p \leq 0.05$

AST and ALT were significantly higher among the two groups when compared to controls ($p \leq 0.001$*), but shooting in AIH. Total bilirubin is significantly increased among the two studied groups. PT is significantly decreased among the two study groups ($p \leq 0.001$*) (table3).

**Table 3: Comparison between the three studied groups according to liver function**

| | Chronic HCV (n=30) | Autoimmune (n=30) | Control (n=20) | Test of Sig. | p |
|---|---|---|---|---|---|
| **ALT(U/l)** | | | | | |
| Min. – Max. | 59.0 – 190.0 | 230.0 – 700.0 | 26.0 – 56.0 | H= 69.453* | <0.001* |
| Mean ± SD. | 109.6 ± 43.70 | 444.6 ± 92.31 | 40.45 ± 9.26 | | |
| Median (IQR) | 99.0(68.0 –143.0) | 460.0(410.0–490.0) | 39.50(32.50–47.50) | | |
| **Sig. bet. grps.** | $p_1$<0.001*, $p_2$<0.001*, $p_3$<0.001* | | | | |
| **AST(U/l)** | | | | | |
| Min. – Max. | 32.0 – 80.0 | 156.0 – 460.0 | 18.0 – 31.0 | H= 69.488* | <0.001* |
| Mean ± SD. | 48.33 ± 12.36 | 271.7± 76.28 | 25.45± 4.35 | | |
| Median (IQR) | 44.0(39.0 – 56.0) | 261.0(206.0 – 300.0) | 26.50(22.0 – 29.50) | | |
| **Sig. bet. grps.** | $p_1$<0.001*, $p_2$<0.001*, $p_3$<0.001* | | | | |
| **Total bilirubin** | | | | | |
| Min. – Max. | 0.70 – 2.20 | 0.30 – 6.0 | 0.20– 1.0 | H= 25.699* | <0.001* |
| Mean ± SD. | 1.28 ± 0.50 | 1.23 ± 1.02 | 0.60± 0.26 | | |
| Median (IQR) | 1.15(0.90–1.70) | 1.0(0.80– 1.20) | 0.70(0.3– 0.80) | | |
| **Sig. bet. grps.** | $p_1$=0.187*, $p_2$<0.001*, $p_3$<0.001* | | | | |
| **Albumin** | | | | | |
| Min. – Max. | 2.10 – 5.20 | 2.0– 5.0 | 3.60 – 4.80 | F= 2.875 | 0.062 |
| Mean ± SD. | 3.92± 0.73 | 3.67± 0.63 | 4.08 ± 0.32 | | |
| Median (IQR) | 3.90(3.60– 4.50) | 3.80(3.20– 4.0) | 4.10(3.85– 4.20) | | |
| **PT** | | | | | |
| Min. – Max. | 58.0– 95.0 | 40.0 – 100.0 | 88.0– 111.0 | F= 30.946* | <0.001* |
| Mean ± SD. | 79.60 ± 10.08 | 75.93 ± 11.47 | 97.30 ± 5.64 | | |
| Median (IQR) | 80.0(74.0– 88.0) | 77.0(74.0– 80.0) | 98.5(92.50– 100.5) | | |
| **Sig. bet. grps.** | $p_1$=0.320, $p_2$<0.001*, $p_3$<0.001* | | | | |

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
p: p value for comparing between the studied groups
p1: p value for comparing between Chronic HCV and Autoimmmune p2: p value for comparing between Chronic HCV and Control
p3: p value for comparing between Autoimmmune and Control
*: Statistically significant at $p \leq 0.05$

ANA, ASMA, AMA, AL/KM and pANCA were significantly positive in AIH when compared to HCV clinical cases ($p \leq 0.001$*). ANA, AMA were positive in 100% of AIH cases, followed by ASMA (90%), AL/KM (66.7%) and pANCA(63.3%) (table 4)

**Table 4: Comparison between the two studied groups according to different autoantibodies**

| | Chronic HCV (n=30) | | Autoimmune (n=30) | | Test of Sig. | p |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **ANA** | | | | | | |
| Negative | 24 | 80.0 | 0 | 0.0 | $\chi^2$= | <0.001* |
| Positive | 6 | 20.0 | 30 | 100.0 | 40.0* | |
| Min. – Max. | 0.07– 0.15 | | 0.07– 0.49 | | | |
| Mean ± SD. | 0.09±0.03 | | 0.19±0.09 | | U= | 0.001* |
| Median (IQR) | 0.07 (0.07– 0.10) | | 0.15 (0.10– 0.26) | | 19.500* | |
| **ASMA** | | | | | | |
| Negative | 23 | 76.7 | 3 | 10.0 | $\chi^2$= | <0.001* |
| Positive | 7 | 23.3 | 27 | 90.0 | 27.149* | |
| Min. – Max. | 0.06– 0.10 | | 0.10– 0.26 | | | <0.001* |
| Mean ± SD. | 0.07±0.02 | | 0.16±0.05 | | U= | |
| Median (IQR) | 0.06 (0.06– 0.07) | | 0.15 (0.15– 0.15) | | 2.500* | |
| **AMA** | | | | | | |
| Negative | 26 | 86.7 | 0 | 0.0 | $\chi^2$= | <0.001* |
| Positive | 4 | 13.3 | 30 | 100.0 | 45.882* | |
| Min. – Max. | 0.06– 0.06 | | 0.10– 0.49 | | | <0.001* |
| Mean ± SD. | 0.06±0.0 | | 0.15±0.10 | | U=0.0* | |
| Median (IQR) | 0.06 | | 0.10 (0.10– 0.15) | | | |
| **ALKM** | | | | | | |
| Negative | 28 | 93.3 | 10 | 33.3 | $\chi^2$= | <0.001* |
| Positive | 2 | 6.7 | 20 | 66.7 | 23.254* | |
| Min. – Max. | 0.06– 0.06 | | 0.10– 0.15 | | | 0.009* |
| Mean ± SD. | 0.06±0.0 | | 0.12±0.03 | | U=0.0* | |
| Median (IQR) | 0.06 | | 0.10 (0.10– 0.15) | | | |
| **pANCA** | | | | | | |
| Negative | 25 | 83.3 | 11 | 36.7 | $\chi^2$= | <0.001* |
| Positive | 5 | 16.7 | 19 | 63.3 | 13.611* | |
| Min. – Max. | 0.07– 0.07 | | 0.07– 0.10 | | | 0.075 |
| Mean ± SD. | 0.07 ± 0.0 | | 0.08 ± 0.01 | | U= | |
| Median (IQR) | 0.07 | | 0.10 (0.07– 0.10) | | 22.500 | |
| **ACA** | | | | | | |
| Negative | 23 | 76.6 | 21 | 70.0 | $\chi^2$= | 0.559 |
| Positive | 7 | 23.3 | 9 | 30.0 | 0.341 | |

$\chi$2: Chi square test. U:Mann Whitney test

p: p value for comparing between the studied groups *: Statistically significant at p ≤ 0.05

Regarding distribution of HLA DRB1 alleles among the 3 different studied groups (table 5) ; a high statistical significance was detected between DRB 1*7, DRB 1*11 and chronic HCV group in comparison to controls and AIH (p ≤ 0.001*).

**Table 5: Distribution of HLA DRB1 alleles in the 3 different studied groups**

| DRB | Chronic HCV (n=30) | | Autoimmune (n=30) | | Control (n=20) | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | No. | % | No. | | |
| DRB 1*1 | 1[a] | 3.3 | 0[a] | 0.0 | 7[b] | 35.0 | 14.585* | MCp<0.001* |
| DRB 1*2 | 1[a] | 3.3 | 2[a] | 6.7 | 8[b] | 40.0 | 12.692* | MCp=0.001* |
| DRB 1*3 | 0[a] | 0.0 | 20[b] | 66.7 | 10[b] | 50.0 | 30.222* | <0.001* |
| DRB 1*4 | 0[a] | 0.0 | 5[b] | 16.7 | 10[c] | 50.0 | 19.829* | <0.001* |
| DRB 1*5 | 1[a] | 3.3 | 16[b] | 53.3 | 1[a] | 5.0 | 26.189* | <0.001* |
| DRB 1*6 | 0 | 0.0 | 0 | 0.0 | 2 | 10.0 | 3.959 | MCp=0.061 |
| DRB 1*7 | 19[a] | 63.3 | 0[b] | 0.0 | 0[b] | 0.0 | 41.530* | <0.001* |
| DRB 1*8 | 3 | 10.0 | 2 | 6.7 | 1 | 5.0 | 0.526 | MCp=0.882 |
| DRB 1*9 | 6 | 20.0 | 2 | 6.7 | 1 | 5.0 | 3.125 | MCp=0.281 |
| DRB 1*10 | 7[a] | 23.3 | 2[ab] | 6.7 | 0[b] | 0.0 | 6.652* | MCp=0.036* |
| DRB 1*11 | 11[a] | 36.7 | 0[b] | 0.0 | 0[b] | 0.0 | 19.501* | MCp<0.001* |
| DRB 1*12 | 1 | 3.3 | 2 | 6.7 | 0 | 0.0 | 1.231 | MCp=0.780 |
| DRB 1*13 | 3 | 10.0 | 2 | 6.7 | 0 | 0.0 | 1. 804 | MCp=0.433 |
| DRB 1*14 | 1 | 3.3 | 2 | 6.7 | 0 | 0.0 | 1.231 | MCp=0.780 |
| DRB 1*15 | 4[a] | 13.3 | 0[b] | 0.0 | 0[ab] | 0.0 | 5.113* | MCp=0.039* |

$\chi$2: Chi square test MC: Monte Carlo
p: p value for comparing between the studied groups *: Statistically significant at $p \leq 0.05$
Common letters are not significant (i.e. Different letters are significant)

DRB1*3 and DRB1*5 were the predominant alleles in AIH (representing 66.7% and 53.3% respectively)
DRB1*3 was detected significantly higher in the AIH group than HCV group. Whereas DRB1*5 was significantly higher in the AIH compared to HCV and control groups ($p \leq 0.001$*).

## DISCUSSION

Hepatitis C virus, a hepatotropic as well as a lymphotropic virus, is linked to various diseases and syndromes. In the majority of infected individuals, HCV infection tends to become chronic due to inability of the immune system to produce a successful response against the virus [19]. Like HCV infection, AIH also has a tendency for chronicity, it is distinguished by the continuous inflammation of hepatocytes and necrosis and a propensity to advance to cirrhosis. Autoantibodies against liver and non-liver antigens are frequently present [20].

This current work is a comparative study between clinical cases of HCV infection and AIH regarding blood picture, liver functions and abundance of autoantibodies. Also, to find out the genetic association of (HLA class II alleles : DRB1*01* - DRB1*15*) and cases of HCV and cases of AIH.

In the current study, as regard patients' sex, (90%) of the patients with AIH were of female gender while in HCV cases the majority of the patients were of male gender (80%). This is consistent with other studies, as vom Steeg LG et al[21] who stated that; by far, both the intensity (i.e., pathogen load within an individual) and prevalence (i.e., number of infected individuals within a population) of infections are often higher for males than females. AIH predominantly affects females, this was mainly attributed to the female sex hormones whose effect was also observed in other autoimmune diseases[22].

Regarding blood picture, the two patient groups demonstrated a significant decline in the platelet count and in haemoglobin concentration. AST and ALT were significantly high among the two studied groups compared to controls, but the liver enzymes levels were shooting in AIH cases. Total bilirubin was significantly increased among the two patients groups, while the PT was significantly decreased, denoting the insult occurring in the hepatocytes and the declining hepatic functions.

Autoantibodies namely; ANA, ASMA, AMA, AL/KM and pANCA were siginificantly positive in AIH compared to HCV cases. ANA, AMA were positive in 100% of AIH cases, then ASMA (90%), AL/KM (66.7%) and pANCA(63.3%). On the contrary, ACA didn't show significant difference among AIH and HCV clinical cases which is consistent with other studies were ACA was the least autoimmune marker to have a positive predictive value in AIH cases[23,24].

HCV infection mount a diversity of clinical presentations. Few individuals will have the ability to clear the virus whereas the majority of the infected will have persistent viremia with a course that ranges from minimal inflammation to progressive cirrhosis up to HCC [25]. Although the exact pathogenesis of HCV

infection is not yet fully defined; but a strong, sustained multispecific CD4 proliferative T cell response is essential for viral clearance in HCV-infected patients [26]. Also a research done on previously HCV-immune chimpanzees found that direct CD4 depletion resulted in chronic evolution in these chimpanzees [27]. These findings suggest that CD4 T cells have a critical role in the outcome of HCV infection and subsequently, HLA class II molecules was given special attention due to its influence on CD4 T cell response [29-31].

In the present study, DRB1*7, DRB1*11, DRB 1*15 were significantly associated with HCV clinical cases compared to AIH group and controls ($p<0.001*$, $0.001*$, 0.039*). These findings were in agreement with other studies done elsewhere like Harris et al. in 2008 and El-Bendary et al. in 2019 [32,33] who claimed that DRB1∗07 is the allele associated with persistence of HCV and non response to interferon. Also, we observed that DRB1*3 , DRB 1*4 alleles were found only in AIH and controls but never in HCV infection group, this may signify a protective role of these alleles (DRB1*3 , DRB1*4). These results were also similar to findings of Shalaa et al research, which was done on Egyptian patients few years ago [34]. Conversely, researchers like Yenigun and Durupinar (2002) [35] who considered DRB1*11 as a protective allele and linked it with self limiting outcome as the body is cleared of the virus spontaneously. This discrepancy may be attributed to sample size, selection bias, different ethnic groups or may be genuine.

The suggested pathogenesis of AIH requires the interaction between genetic predisposition and environmental triggers. The genetic predisposition may be related to defects in immunologic control of autoreactivity and the environmental agent triggers the autoimmune response against liver-specific antigens, causing necroinflammatory damage, fibrosis, and, eventually, cirrhosis. An epidemiological study done in recent years, revealed the concurrence of AIH in identical twins and also found AIH aggregation in certain families. Furthermore, AIH patients were frequently diagnosed with other extrahepatic diseases related to autoimmunity. These findings suggest common genetic risk factors for AIH and other auto-immune diseases. The HLA is considered the strongest genetic risk factor for AIH [36].

The HLA is considered the strongest genetic risk factor for AIH, several studies were done around the globe to capture the culpable alleles. The DRB1*04:04 and DRB1*04:05 alleles were associated with cases of AIH in Japan and Korea, whereas the DRB1*03:01 and DRB1*04:01 alleles were incriminated in European studies. As in Latin America , DRB1*13:01 was linked to vulnerability to AIH [37]. In the search for safe alleles, DRB1*15:01 was considered protective in Japan and North America [37,38].

Among our AIH cases DRB1∗03 and DRB1∗05 were the main alleles representing 66.7% and 53.3% respectively, this result was statistically significant ($p<0.001*$, $0.001*$). Shalaa et al[34] and other researchers recorded similar findings. We also reported that neither of our AIH cases carried DRB1*15 allele, supporting the claim by other researchers that this allele is protective against AIH [36].

## CONCLUSION

We can conclude that certain HLA alleles of class II could be linked to cases of hepatitis (either caused by HCV or autoimmune) and can predict clinical course of the illness and outcome of the disease and these alleles differ according to several factors including the geographic distribution and ethnic background.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

**Acknowledgment**

We would like to acknowledge Alexandria Main Armed Forces Hospital, Egypt who sponsored materials and equipment used in our current study.

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