Original Article

Human Leukocyte Antigen Class I Alleles can Predict Response to Pegylated Interferon/Ribavirin Therapy in Chronic Hepatitis C Egyptian Patients

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Abstract

Background: Racial differences and broad spectrum response to anti-hepatitis C (anti-HCV) therapy suggest a possible role for host genetic diversity in treatment outcomes. We aim to determine the association and predictive value of certain human leukocyte antigen (HLA) class I alleles with either susceptibility to viral clearance or persistence following pegylated interferon (Peg-IFN) plus ribavirin therapy in chronic hepatitis C (HCV) genotype 4 patients in Egypt.

Methods: This study included 200 unrelated chronic HCV patients who received Peg-IFN plus ribavirin therapy [112 patients with sustained virological response (SVR) and 88 non-responders (NR)]. Serological testing of HLA class I antigens (HLA-A and HLA-B alleles) were performed by standard complement-dependent microlymphocytotoxicity assay.

Results: The frequency of HLA-A01 was significantly higher in SVR than in NR cases [OR: 0.51; 95% CI: 0.27–0.981; *P* = 0.042], while the frequency of alleles B38 (*P* = 0.011), B40 (*P* < 0.001) and B41 (*P* < 0.001) was significantly higher in NR cases (OR/95% CI: 7.05/(1.39–18.01), 10.31/3.14–36.1 . On logistic regression analysis, presence of the HLA-A01 allele was associated with SVR (OR: 0.50; 95% CI: 0.28–0.89; *P* = 0.02) and HLA-B38 can predict non response to therapy (OR: 7.92; 95% CI: 1.67–37.54; *P* = 0.009) with an overall accuracy of 60%.Severe fibrosis (OR: 3.035; 95% CI: 1.521–6.091; *P* = 0.002), high viremia (OR: 2.69; 95% CI: 1.11–6.53; *P* = 0.005) and steatosis (OR: 2.1; 95% CI: 1.002–3.90; *P* = 0.041) predicted no response with an overall accuracy of 81.8%.

Conclusion: HLA-A01 and HLA-B38 alleles are associated with and may have a role in the outcome of response to Peg-IFN plus ribavirin therapy in Egyptian patients diagnosed with chronic HCV infection. The use of immunologic markers to predict the outcome of treatment may help pharmacogenetic personalization of treatment for HCV infection.

Keywords: Fibrosis, HCV, HLA alleles, Peg-IFN/Ribavirin therapy, treatment response

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Introduction

Chronic hepatitis C (HCV) infection is a major cause of end-stage liver disease and liver cancer worldwide.[1] HCV treatment has been transformed over the last decade by the use of improved therapy regimens, including pegylated interferon (Peg-IFN), as either monotherapy or in combination with the nucleoside analog ribavirin.[2,3] Even with the recent combination therapy of Peg-IFN plus ribavirin for chronic HCV infection, viral response is variable and only half of those treated could clear the virus.[4]

The racial difference and the broad spectrum of response to therapy suggest a possible role for host genetic diversity in the response to anti-HCV therapy. Genes encoding the human leukocyte antigens (HLA) are found in the major histocompatibility (MHC) complex region of chromosome 6, and are critical in the regulation and initiation of the cellular immune response. MHC molecules present foreign antigens to T-cell receptors bearing $CD8^+$ and $CD4^+$ T-lymphocytes, respectively.[5] On the other hand, IFN-α has been identified as an immunomodulatory cytokine that induces T-lymphocyte activation and expression of MHC molecules.[6] Numerous class I and class II HLA polymorphisms appear to respond to IFN-based therapy. Although several strong associations between HLA alleles and the natural history of infectious agents have been reported, few studies have examined the association between HLA polymorphism and response to Peg-IFN plus ribavirin therapy. This study aims to investigate the association and predictive value of certain HLA class I alleles with susceptibilities to viral clearance or persistence after Peg-IFN plus ribavirin therapy in chronic HCV patients who reside in Egypt.

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Materials and Methods

Sample size and study power

We used the MedCalc program to calculate the sample size. The confidence level of our study was 95% with an alpha error of 0.05. The power of this study was decided at 90 with a beta error of 10. The maximum prediction of response by HLA was 60% with the minimal prediction from 50%. Our estimated sample was 112 patients, however we increased the sample by more than one third

to guard against drop out thus, we enrolled 200 patients.

Patients

Included in this study were 200 unrelated chronic HCV Egyptian patients who received Peg-IFN plus ribavirin therapy during the period between January 2008 and December 2010. The study was conducted in the Unit of Tropical Medicine, at Mansoura University Hospital. Patients were selected according to the International Program Guideline selection criteria with the following inclusion criteria: age 18–60 years; HCV RNA-positive serum; compensated liver disease (total serum bilirubin < 1.5 mg/dL; INR = 1.5; serum albumin ≥ 3.5gm/dL; platelet count ≥ 90000 mm; no evidence of hepatic decompensation (hepatic encephalopathy, esophageal varices, or ascites); acceptable hematological and biochemical indices (hemoglobin ≥ 13 g/dL for men and 12 g/dL for women; neutrophil count > 1500/mm^3; serum creatinine < 1.5 mg/dL); and body mass index (BMI) < 30.

Exclusion criteria

Patients positive for hepatitis B (HBsAg) or human immunodeficiency virus infections, the presence of a major uncontrolled depressive illness, solid organ transplant (renal, heart, or lung), autoimmune hepatitis or other autoimmune condition known to be exacerbated by Peg-INF and ribavirin (autoimmune hemolytic anemia), untreated thyroid disease, pregnant or unwilling to comply with adequate contraception, severe concurrent medical disease such as severe hypertension, heart failure, significant coronary heart disease, poorly controlled diabetes, chronic obstructive pulmonary disease, and history of previous treatment with IFN-ribavirin therapy were the exclusion criteria.

Informed consents were obtained from subjects included in the study. The study was approved by the Ethical Committee of Mansoura Faculty of Medicine, Egypt, in accordance with the Ethical Standards Guidelines within the Declaration of Helsinki.

Patients were given weekly fixed doses (180 μg) of Peg-IFN α 2a by subcutaneous injections. All received ribavirin in an adjusted dose according to body weight; patients < 75 kg received 1000 mg and those ≥ 75 kg received 1200 mg. Initial responders continued therapy for a total duration of 48 weeks. We excluded patients who relapsed or those who underwent Peg-IFN and/or ribavirin dose reductions. Thus, the study included a total of 112 patients who achieved sustained virological response (SVR), which was defined as normalization of liver enzymes and negative HCV RNA PCR at the end of therapy and for six months following cessation of treatment in addition to 88 non-responder (NR) patients who failed to attain a negative HCV RNA at week 24 from the start of treatment or a decline in HCV RNA of ≥2 log10 IU/mL at week 12 of treatment. Blood samples were obtained from all subjects. Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were determined by a Hitachi 902 instrument. We tested serum samples for anti-HCV Ab by using third-generation assays (Axysm HCV EIA Test System) and HCV RNA quantification (Stratagene M×3000P Real-Time PCR System with a detection limit of 15 IU/mL) before, during, and after treatment.

Liver histopathology

All patients had liver biopsies that showed evidence of chronic hepatitis within 12 months prior to onset of therapy. Liver histology was graded and staged according to the Ishak Fibrosis Scaling System.[7]

HCV genotyping

A total of 5 mL of patient serum was isolated and separated for RNA extraction and preserved at -70°C. Samples for HCV genotyping were analyzed by the real-time PCR TaqMan SNP genotyping assay according to Ohno et al.[8] We enrolled only those who had genotype 4 HCV in this study.

HLA typing

We obtained 10 mL of heparinized blood from the study patients. Peripheral blood mononuclear cells were isolated using Lymphoflot (Biotest AG, Dreieich, Germany). Serological testing of HLA class I antigens (HLA-A, and HLA-B) for patients and controls were performed with a standard complement-dependent microlymphocytotoxicity assay. This detection method uses well-characterized HLA antisera that are placed into individual wells on commercial 72-well class I typing trays (Biotest AG, Dreieich, Germany) which are organized as a panel to identify complete HLA types for A and B loci. In the presence of exogenous complement, HLA antibodies are cytotoxic to lymphocytes that express the corresponding antigen. After further incubation, cell death was determined by trypan blue, a vital stain exclusion. Interpretation of the reactivity pattern denoted the subject's HLA type.[9]

Statistical analysis

We used the Statistical Package for Social Sciences (SPSS) version 10 program for statistical analyses. Data were described as mean ± SD for quantitative data, and frequency and proportion for qualitative data.

Data analysis was performed to test for statistically significant differences between groups. For quantitative date we used the student's *t*-test as comparison between the two groups after log transformation. The Chi square test compared qualitative data. Data that were significant according to univariate analysis were then entered in multivariate regression analysis to determine the predictable variable that affected the response. We considered *P* to be significant at < 0.05 with a 95% confidence interval (CI).

Results

Of the 200 study patients, 28 (14%) had evidence of low HCV RNA levels (< 100,000 IU/mL), 104 (52%) had moderate HCV RNA levels (100,000–800,000 IU/mL) and 68 (34%) had high viral HCV RNA levels (>800,000 IU/mL). We observed significant fibrosis in 90 (45%) patients (Fibrosis stage: 3–4) and mild fibrosis in 110 (55%) (Fibrosis stage: 0–2). Six months after completing 48 weeks of Peg-IFN plus ribavirin therapy, 112 (56%) patients achieved SVR while 88 (44%) stopped therapy at either weeks 12 or 24 as they were NR (Table 1).

A comparison of clinical and laboratory parameters between SVR and NR showed statistically significant differences in the frequency of fibrosis, steatosis, and baseline viral load levels (Table 2). Severe fibrosis (OR: 4.01; 95% CI: 2.22–7.26; $P < 0.0001$), presence of steatosis (OR: 1.75; 95% CI: 0.99–3.08; $P = 0.034$), high viral load levels (OR: 10; 95% CI: 2.82–39.4; $P < 0.0001$) were significantly more frequent in NR patients. SVR patients had significantly lower fibrosis scores ($P < 0.0001$), as well as lower ($P < 0.001$) and moderate ($P = 0.039$) baseline viral loads. In our study, we detected 11 alleles at the A locus and 16 alleles at the B locus. HLA-A02 was the most frequent allele among study samples which was present in 110/200 (55%) total cases (SVR +

Table 1. Clinical and demographic data of chronic HCV patients.

Age (years)	
Mean ± SD	41.64 ± 5.76
Range	(28–55)
Gender	
♂	162 (81%)
♀	38 (19%)
Treatment Response	
Non responder	88 (44%)
SVR	112 (56%)
Serum ALT	
One fold	120 (60%)
Two fold increase	24 (12%)
Three fold increase	24 (12%)
Four fold increase	32 (16%)
Viral load	
Low (< 100.000 IU /mL)	28 (14%)
Moderate (100.000–800.000 IU/mL)	104 (52%)
High (> 800.000 IU/mL)	68 (34%)
SVR=sustained virological response	

Table 2. Corellation of clinical and laboratory variables between non-responder (NR) and sustained virological response (SVR) cases.

Variable	NR (N = 88) *n* (%)	SVR (N =112) *n* (%)	OR	95% CI	*P*-value
Gender					
Female	16 (42.4)	22 (57.9)	0.90	(0.44–1.85)	0.47
Male	72 (44.4)	90 (55.6)	1.10	(0.51–2.39)	
Fibrosis					
Significant	56 (62.2)	34 (37.8)	4.01	(2.22–7.26)	0.0001*
Mild	32 (29.1)	78 (70.9)			
Steatosis					
Negative	50 (51)	70 (47.9)	1.75	(0.99–3.08)	0.034*
Positive	38 (37.3)	42 (77.8)			
Activity					
Severe	76 (52.1)	70 (47.9)	3.8	(1.85–7.78)	0.056
Mild	12 (22.2)	42 (46.4)			
Viral Load					
Low (<100,000 IU /mL)	6 (15.4)	22 (84.6)			
Moderate (100,000-800,000 IU/mL)	38 (36.5)	66 (63.5)	3.17	(0.93 – 11.78)	0.039*
High (>800,000 IU/mL)	44 (64.7)	24 (35.5)	10	(2.82–39.4)	0.001*
Viral load*					
Median	80.64	36.1			0.001*
Range	(5.2–1000)	(0.34–777.93)			

OR = odds ratio; CI = confidence interval, *P<0.05 is significant. Students' t-test was used after log transformation for viral load.

NR) and in 60/112 SVR cases, however there was no statistical difference between SVR and NR cases. As seen in Table 3 the frequency of HLA-A01 was significantly higher in SVR compared to NR (OR: 0.51; 95% CI: 0.27–0.981; $P = 0.042$). The same was observed for class I alleles (B05, B12) which were the most frequent alleles found in 66/200 (33%), each for SVR and NR cases. This frequency was not statistically significant between SVR and NR cases. NR had significantly higher frequencies of B38 ($P = 0.011$), B40 ($P < 0.001$) and B41 ($P < 0.001$) alleles (OR/95% CI were: 7.05/ (1.39–18.01), 10.31/3.14–36.1). Interestingly all patients who carried the B41 allele were NR (Table 4).

According to logistic regression analysis, the presence of the HLA-A01 allele might have a limited role in prediction of SVR (OR: 0.504; 95% CI: 0.283–0.89; $P = 0.02$), whereas the presence of the HLA-B38 allele has a 7.92-fold risk of non-response to Peg-IFN plus ribavirin therapy in chronic HCV patients (OR = 7.92; 95% CI: 1.67–37.54; $P = 0.009$) with a constant -1.108, and an overall accuracy of 60%. With regards to clinical parameters, severe fibrosis (OR: 3.035; 95% CI: 1.521–6.091; $P = 0.002$), high histological activity (OR: 2.69; 95% CI: 1.11–6.53; $P = 0.028$), moderate viremia (OR: 2.74; 95% CI: 1.34-5.57; $P = 0.005$), high viremia (OR: 9.39; 95% CI: 2.61–38.75; $P < 0.001$), and presence of steatosis (OR: 2.1; 95% CI: 1.002–3.90; $P = 0.041$) predicted non-response to Peg-IFN plus ribavirin therapy in chronic HCV patients with a constant of -2.746 and overall accuracy of 81.8% (Tables 5 and 6).

Discussion

In the present study we investigated the possible association and predictive value of certain HLA class I alleles and susceptibilities to viral clearance or persistence after Peg-IFN plus ribavirin therapy in chronic HCV patients. According to logistic regression analysis, the presence of the HLA-A01 allele was independently

Table 3. HLA-A alleles and response to pegylated interferon (Peg-IFN) plus ribavirin in chronic HCV patients.

Allele	N	Non-responder (NR) N = 88	Sustained Viological Response (SVR) N = 112	OR	95% CI	*P*-value
A01	70	22	48	0.51	(0.27–0.981)	0.042*
A02	110	50	60	1.14	(0.63–2.08)	0.75
A03	58	30	28	1.55	(0.8–3)	0.21
A09	16	4	12	0.40	(0.1–1.39)	0.18
A11	58	32	26	1.85	(0.95–3.59)	0.0711
A23	4	2	2	1.28	(0.13–13.01)	0.79
A24	2	2	0	—	—	0.37
A25	2	2	0	—	—	0.37
A30	20	8	12	0.83	(0.29–2.22)	0.88
A31	4	0	4	—	—	0.19
A32	2	0	2	—	—	0.58

OR = odds ratio; CI = confidence interval. *$P < 0.05$ is significant.

Table 4. HLA-B alleles and response to pegylated interferon (Peg-IFN) plus ribavirin in chronic HCV patients according to response.

Allele	N	Non-responder (NR) N = 88	Sustained Virological Response (SVR) N = 112	OR	CI	*P*-value
B02	4	2	2	1.28	(0.13–13.01)	0.79
B05	66	28	38	0.91	(0.48–1.72)	0.87
B07	2	2	—	—	—	0.37
B08	2	2	—	—	—	0.37
B12	66	28	38	0.91	(0.48–1.72)	0.87
B13	26	8	18	0.52	(0.20–1.36)	0.21
B14	2	—	2	—	—	0.58
B15	12	8	4	2.70	(0.70–11.1)	0.18
B17	30	18	12	2.14	(0.91–5.09)	0.086
B18	8	2	6	0.41	(0.06–2.33)	0.45
B21	12	2	10	0.24	(0.03–1.2)	0.09
B27	14	4	10	0.49	(0.12–1.77)	0.35
B35	40	18	22	1.05	(0.50–2.23)	0.88
B38	12	10	2	7.05	(1.39–18.01)	0.011*
B40	28	24	4	10.31	(3.14–36.1)	< 0.001*
B41	22	22	—	—	—	< 0.001*

OR = odds ratio; CI = confidence interval. *$P < 0.05$ is significant.

Table 5. Logistic regression to predict non-responder (NR) to peg interferon (Peg-INF) in HCV patients by HLA-A and B alleles.

Allele	B coefficient	*P*-value	OR	95% CI
HLAA				
A01	-0.686	0.02*	0.504	0.283–0.89
HLAB				
B17	0.602	0.127	1.82	0.84–3.95
B38	2.07	0.009*	7.92	1.67–37.54
B40	0.001	0.99	1.001	0.45–2.203
B41	0.014	0.97	1.014	0.42–2.43
B21	-1.319	0.093	0.26	0.056–1.249

OR: Odds ratio; CI: Confidence interval, constant -1.108, with overall accuracy of 60%. *$P<0.05$ is significant.

Table 6. Logistic regression to predict non-responder (NR) to peg-interferon (Peg-IFN) in HCV patients by clinical parameters.

Factor	B coefficient	*P*-value	OR	95% CI
Age	0.025	0.41	1.026	(0.96–1.09)
ALT > 2-fold	0.902	0.187	1.99	(0.64–9.39)
Severe fibrosis	1.11	0.002*	3.035	(1.521–6.091)
High activity	0.911	0.028*	2.69	(1.11–6.53)
Moderate viremia	1.008	0.005*	2.74	(1.34–5.57)
High viremia	2.24	0.001*	9.39	(2.61–38.75)
Steatosis	0.682	0.041*	2.1	(1.002–3.90)

OR = odds ratio; CI = confidence interval, constant -2.746, with overall accuracy 81.8%. *$P < 0.05$ is significant.

associated with SVR whereas carriers of HLA-B38 have a 7.92-fold risk of nonresponse to Peg-IFN plus ribavirin therapy in chronic HCV patients. Although the class I allele A02 was the most frequent allele in our study population, its frequency was not statistically different between SVR and NR which was in accordance with a large study conducted on 373 SVR cases who were African or Caucasian Americans. The study observed that HLA-A02 carriers were significantly more prevalent in SVR [(Relative Risk = 1.33 (1.08–1.64); $P = 0.008$)].[5] The limitation of the American study was that it only screened for HLA alleles within SVR

patients. There was no comparison between carriers of the same alleles in NR as performed in the current study.

Regarding the genetic background, it may be presumed that a potential immunological response could be significantly enhanced by Peg-IFN plus ribavirin therapy in chronic HCV patients. Effective presentation of viral antigens to CD4+ and CD8+ T-cells by HLA class II and class I molecules, respectively, is the key for regulation of an optimal immune response against viral infection. With the upregulated expression of immunogenetic molecules which enhances the immune response by IFN, genetic variations at the HLA loci with respect to antigen presentation might be related to a response to IFN-based therapy.[10] Assessment of the association between response to Peg-IFN plus ribavirin and HLA-A01 and HLA-B38 by detection and quantification of these alleles may help in the optimization of treatment. If HLA-B38 is a negative predictor of response to treatment, more aggressive therapeutic regimens (by adding protease inhibitors or extending the duration of therapy) for patients who carry the alleles may be suggested. The possibility of individualizing dose and duration of Peg-IFN plus ribavirin therapy makes it possible to individualize treatment according to baseline predictors.

The association between specific HLA alleles and antiviral response to conventional IFN monotherapy has been investigated in numerous studies. In Japanese patients the HLA–A24-B-54-DR4 haplotype and HLA B-54 allele were predictors of poor response ($P = 0.0378$, $P = 0.0258$, respectively), whereas HLA-B55-B62-Cw3, HLA-B51,HLA-CW1, and HLA B 7-DRB10101 haplotypes were identified as predictors of good response to conventional IFN monotherapy[6,11–13]

In Spanish populations HLA B-44 was associated with responsiveness to IFN and ribavirin therapy (OR: 4.84; 95% CI: 1.3–17.8; $P = 0.017$). However, HLA B44+ was not associated with SVR in patients treated with IFN alone in chronic HCV cases.[14] In a Turkish study, the HLA B-13 allele was associated with nonresponse to IFN monotherapy, 50% of NR cases were HLA DRB1*13 positive, yet only 7% were positive in the responder group ($P < 0.05$).[15]

In Taiwanese chronic HCV patients, HLA-A11-B51, CW15, and DRB-15 alleles were associated with SVR, whereas while HLA-A24 was associated with poor response to IFN monotherapy.[16] Recently, Peg-IFN plus ribavirin has become the standard of care for treatment of chronic HCV patients. A recent study on Taiwanese chronic HCV patients treated with Peg-IFN plus ribavirin, the same HLA-A24 allele was significantly associated with SVR. Although the study found that no single HLA class II allele was associated with response to therapy, analyses of the haplotypes showed that B40-DRB1*3, B46-DRB1*9, Cw1- DQB1*3, and Cw1- DRB1*9 might be associated with non-response.[10] The addition of ribavirin or the different sample size can explain the opposite effect of HLA-A24 carriage on IFN mono- or combined therapy. Ribavirin possesses activity against several RNA and DNA viruses. It has been suggested that ribavirin modulates the immune system by promoting T helper cell (TH) 1 over the TH2 phenotype.[17] To our knowledge, the only data published from Egypt is the association between HLA DR2 with response to conventional IFN therapy. In this study, 55 patients received IFN-α therapy for six months.[18]

The present study showed an association between certain HLA alleles to response in patients with chronic HCV in addition to other clinical, virological, and histological factors. Severe fibrosis and steatosis were predictors of non-response to Peg-IFN plus ribavirin therapy in this study. These results agreed with numerous previous studies on both genotypes 1 and 4, which noted that patients with advanced fibrosis or cirrhosis showed marked decreases in SVR.[19–22] According to other studies important histological parameters associated with SVR were the absence of advanced fibrosis and steatosis.[23–27]

Although serum HCV RNA levels do not predict the degree of liver injury, the assessment of viral load pretreatment during and after therapy is an important tool for predicting treatment outcome. Low viral load has been identified as an independent predictor of SVR response in many studies in addition to our study.[25,28–34] In conclusion, HLA-A01 and HLA-B38 alleles are associated with and may predict the outcome of Peg-IFN plus ribavirin therapy in Egyptian patients who have chronic HCV. Extensive study of genetic polymorphisms as modulators of HCV treatment may help select candidates for therapy and optimize the response to Peg-IFN plus ribavirin by pharmacogenetic personalization of treatment for HCV. The use of immunologic markers to predict the outcome of treatment would reduce treatment of NR and assist with identification of those in whom therapy would be justified.

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