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Effect of human leukocyte antigen class I and II alleles on hepatitis C viral load among chronic hepatitis C patients in Southern Taiwan

Kuo-Chih Tseng [a,b], Chih-Wei Tseng [a,b,c,d], Yu-Hsi Hsieh [a,b], Chin-Kuo Chang [e], Ning-Sheng Lai [a,b], Tsung-Hsing Hung [a,b], Ting-Tsung Chang [f,g,*]

[a] Department of Internal Medicine, Buddhist Dalin Tzu Chi General Hospital, Chia-Yi, Taiwan
[b] School of Medicine, Tzuchi University, Hualien, Taiwan
[c] Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan
[d] School of Medicine, National Yang-Ming University, Taipei, Taiwan
[e] Institute of Psychiatry, Department of Health Service and Population Research, King's College, London, UK
[f] Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan
[g] College of Medicine, National Cheng Kung University, Tainan, Taiwan

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ABSTRACT

The viral load of hepatitis C virus (HCV) in chronic hepatitis C patients affects clinical outcomes and response to interferon treatment. Various factors may be involved in determining the viral load, including host genetic factors. The aim of this study was to investigate the relationship between HCV viral load and human leukocyte antigen (HLA) class I and class II alleles. One hundred and six HCV RNA positive subjects were enrolled, and viral load was measured. HLA-A, -B, -C, -DR, and -DQ loci were determined by sequence-based genotyping. Univariate analysis indicated that HLA-B*40 and HLA-C*07 alleles had significantly higher HCV RNA levels ($P < 0.05$). Patients with the HLA-C*15 allele exhibited a trend toward a lower HCV viral load ($P = 0.06$). After controlling for confounding factors, multivariate analysis revealed that only HLA-C*15 allele was identified as a significant determinant for HCV-RNA level (slope = −0.91, 95% CI: −1.58, −0.24; Holm's $P < 0.01$). Patients expressing the HLA-C*15 allele had significantly lower HCV RNA levels. HCV genotype 1 was significantly associated with high HCV RNA levels ($P < 0.05$ by Mann–Whitney *U* test). In conclusion, HLA-C*15 is an important host immunogenetic factor with an inverse association to HCV viral load in CHC patients in Taiwan. HCV genotype 1 is the viral factor that associated with high viral load.

1. Introduction

The hepatitis C virus (HCV) is a small, enveloped RNA virus that belongs to the family *flaviviridae* and genus *hepacivirus* [1]. HCV's genome is 9600 nucleotides in length. At present, there have been 6 genotypes and more than 90 subtypes identified around the world. After HCV infection, approximately 55–85% of patient's immune systems cannot eliminate the virus and, thus, chronic hepatitis C (CHC) develops [1]. CHC is often asymptomatic but is usually associated with fluctuating or persistent elevated alanine aminotransferase (ALT) levels. Major long-term complications of CHC include cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC) [1]. Retrospective-prospective studies on the long-term natural history of HCV infection have demonstrated that 15–20% of CHC patients develop cirrhosis within 30 years of HCV infection [2,3]. Once cirrhosis has occurred, the annual risk of HCC, hepatic decompensation and liver-related death is approximately 1–4%, 5% and 2–4%, respectively [2,4–7].

The risk of transmission of HCV is correlated with the HCV viral load [8]. The HCV viral load was stable in 93.6% patients with CHC infection, whereas only 6.4% of those patients revealed marked HCV viral load fluctuation during 1–2 years of follow-up [9]. Many studies have focused on the relationship between the HCV RNA level and the clinical course of liver disease. Although controversial, most studies have demonstrated that the HCV RNA level is correlated with the severity of liver diseases [10–12]. Furthermore, the HCV RNA level has been identified as a critical predictive factor for the clinical outcome of CHC patients receiving pegylated

Abbreviation: hepatitis C virus, HCV; chronic hepatitis C, CHC; alanine aminotransferase, ALT; hepatocellular carcinoma, HCC; pegylated interferon, PEG-IFN; ribavirin, RBV; human leukocyte antigens, HLA; major histocompatibility complex, MHC.

* Corresponding author at: Department of Internal Medicine, National Cheng Kung University Hospital, 138 Sheng-Li Road, Tainan 704, Taiwan. Fax: +886 6 2743166.
E-mail address: ttchang@mail.ncku.edu.tw (T.-T. Chang).

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interferon (PEG-IFN) plus ribavirin (RBV) therapy [2]. Additional factors that may affect HCV viral load and outcomes in CHC patients are being investigated.

In addition to viral, environmental, and behavioral factors, the host's genetic diversity contributes to the spectrum of clinical outcomes in HCV infection. Human leukocyte antigens (HLAs) are encoded by genes within the major histocompatibility complex (MHC) region on the short arm of chromosome 6. The HLA complex is highly polymorphic with multiple alleles at linked loci. The expression of a particular HLA allele may modify the presentation of a processed antigen to the T cell receptor and, consequently, affect immune responses [13,14]. Thus far, studies have investigated the role of HLA polymorphisms in determining self-limiting versus persistent CHC infections [15–22]. Two specific HLA class II alleles (DQB1*03:01 and DRB1*11) have been shown to be associated with self-limiting HCV infection in predominantly Caucasian populations [22]. Furthermore, Wang et al. studied the relationship between HLA and HCV viral load and suggested that a host's immunogenetic factors significantly influence the HCV viral load during CHC infection in the eastern Taiwanese population [23]. Therefore, the aim of this study was to assess the effect of HLA class I and II alleles on the HCV viral load in a CHC population in Southern Taiwan.

2. Subjects and methods

2.1. Subjects

The study subjects were a cohort of CHC patients receiving PEG-IFN plus RBV [24]. In brief, CHC patients who had completed the combination therapy of PEG-IFN alpha 2a or 2b plus RBV for 6 months were recruited at the Dalin Tzu Chi General Hospital in Southern Taiwan between June 2005 and April 2007. The inclusion criteria were seropositivity for anti-hepatitis C antibody for at least 6 months, detectable serum HCV RNA level at entry, and serum ALT greater than one time of the upper normal limit. The exclusion criteria were any malignant neoplasm, decompensated liver disease, autoimmune diseases, alcohol abuse, positive hepatitis B surface antigen, HIV infection, neutropenia (<1500 neutrophils/ml), thrombocytopenia (<75,000 platelets/ml), anemia (<12 g of hemoglobin/dl in females and <13 g/dl in males), and poorly controlled psychiatric disorders. The study was approved by the Ethics Committee of Dalin Tzu Chi General Hospital (approval number B09601022). All patients signed the informed consent with full study information and instructions.

2.2. HCV quantification and genotyping

Serum HCV RNA was quantified by real-time polymerase chain reaction technology [25]. The lower limit of detection was 86 copies/mL. HCV genotyping was determined by nested polymerase chain reaction and melting curve analysis (Roche LightCycler; Biotronics Tech Corp., Lowell, MA, USA) [26].

2.3. HLA typing

Genomic DNA was extracted from serum samples using the QIAamp DNA blood mini kit (Qiagen Inc., Valencia, CA, USA). HLA class I and class II alleles were determined by a DNA typing technique using sequence-specific primers (SSP) (HLA-A.B.C.DR.DQ kit; Dynal Allset SSP, Dynal Biotech, Bromborough, Wirral, UK).

2.4. Statistical analysis

Because the major outcome, HCV RNA titer, had a broad range with a positively skewed nature that violated the statistical assumption of normal distribution, statistical descriptions for the patients' demographic and clinical features were performed using medians and interquartile ranges with comparisons of non-parametric methods between and among categories. Using the Mann–Whitney U test, the HCV RNA titers ($\times 10^3$) in patients expressing each HLA class I and II allele were compared to titers in patients without the allele to identify each allele's individual influence on HCV viral load. Furthermore, because HCV viral titer was a continuous variable, we used a linear regression to examine the effects of HLA class I and II alleles as covariates on HCV-RNA titer in separate models to control for potential confounding factors. As previously mentioned, the level of HCV RNA titer did not fit a not normally distribution. A logarithmic transformation with base 10 was performed before fitting the HCV RNA titers into the models as the dependent variable. Regardless of their predictor status in the univariate analyses, age, gender, HCV genotype, BMI, diabetes mellitus, and GPT were considered to be potential confounders. By including the same covariate set in all multivariate analyses, the results from our model were comparable across all HLA classes I and II alleles. Holm's procedure, a step-down modified Bonferroni correction method, was applied to rectify the issue of multiple comparisons on HLA alleles. SPSS 13.0 for Windows was utilized to perform all the statistical analyses, and the significance level (α value) was set as 0.05.

3. Results

During the period between June 2005 and April 2007, 106 chronic hepatitis C patients were enrolled into this study at Dalin Tzu Chi General Hospital in Southern Taiwan. The 59 females and 47 males were on average of 53.08 years old (SD = 11.35). The average HCV RNA level was 1317×10^3 copies/ml. HCV RNA levels are presented in Table 1 and are categorized by demographics and clinical features. HCV genotype 1 was found to be a significant determinant for higher HCV RNA levels ($P < 0.05$ by the Mann–Whitney U test). In Table 2, a univariate analysis showed that patients with the HLA-B*40 and HLA-C*07 alleles had significantly higher HCV RNA levels than patients without the two alleles ($P < 0.05$). In contrast, patients with the HLA-C*15 allele exhibited a trend toward a lower HCV viral load ($P = 0.06$). After controlling for the confounding factors of age, gender, HCV genotype, BMI, and diabetes mellitus, the effect of each HLA class I and class II allele on HCV viral load was analyzed and is presented in Table 3. Only the HLA-C*15 allele was identified to be a significant determinant for the HCV-RNA level (slope = −0.91, 95% CI: −1.58, −0.24; Holm's $P < 0.01$). Thus, HCV RNA titers were significantly decreased in patients expressing HLA-C*15 allele.

4. Discussion

In this study, we showed that CHC patients with the HLA-C*15 allele have significantly lower HCV viral loads than CHC patients without the HLA-C*15 allele (slope = −0.91, 95% CI: −1.58, −0.24; Holm's $P < 0.01$). HLA-C belongs to the MHC class I heavy chain receptors and plays an important role in the regulation of natural killer cell activity through killer cell immunoglobulin-like receptors. Killer cell immunoglobulin-like receptor recognition provides vital information to NK cells about whether a target cell should be lysed or spared [27]. Natural killer cells are important in the innate defense response against hepatitis C viral infections. Previous studies have demonstrated that KIR receptor genes and major histocompatibility complex (MHC) class I and II loci helped determining the clinical outcome of HCV infection [28]. Two studies also demonstrated that the genes encoding the inhibitory NK cell receptor KIR2DL3 and HLA-C1 ligand directly influence the spontaneous clearance of HCV infection in Caucasians and African