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Polymorphism of the IL28B gene (rs8099917, rs12979860) and virological response of Pakistani hepatitis C virus genotype 3 patients to pegylated interferon therapy

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SUMMARY

Background: The gold standard treatment for chronic hepatitis C virus (HCV) infection is pegylated interferon (PEG-IFN) in combination with ribavirin. Most patients treated with PEG-IFN achieve a sustained virological response (SVR). However host genetic factors play a vital role in the spontaneous and treatment-induced clearance of HCV infection from these infected patients. In the current study, polymorphisms of *IL28B* (rs8099917 and rs12979860) were analyzed and their association with the virological response to PEG-IFN alpha treatment was determined.

Methods: One hundred and fifty HCV genotype 3 patients were assessed to study the correlation of *IL28B* with a therapeutic regimen of PEG-IFN alpha plus ribavirin. Twenty patients were excluded due to a refusal to participate in the study and 25 patients failed to meet the inclusion criteria. Of the 105 patients recruited, 49 (46.7%) were male and 56 (53.3%) were female. In order to determine single nucleotide polymorphisms of rs8099917 and rs12979860, the sample was amplified by PCR and then *IL28B* typing was carried out by restriction fragment length polymorphism (RFLP) followed by standard sequencing.

Results: We found three types of genotype in rs8099917 of *IL28B*: wild-type TT in 60.0% of patients, heterozygous GT minor genotype in 36.2%, and GG in 3.8%. The frequency of the CC genotype of rs12979860 was 54.3%, CT was 37.1%, and TT was 8.6%. Overall, SVR was achieved in 68.6% of patients. A higher SVR was achieved for patients with the favorable genotype CC of rs12979860, with 84.2% as compared to 56.4% and 22.2% for minor genotype CT and TT, respectively ($p = 0.0001$). We did not find a significant association for SVR to antiviral treatment in patients with genotype TT (rs8099917) (71.9%, $p = 0.36$). The rapid virological response (RVR) rate was significantly higher in patients with major genotype TT (88.9%, $p = 0.04$). These results show that *IL28B* polymorphism is highly associated with SVR to therapy in the Pakistani population infected with HCV genotype 3.

Conclusions: HCV-infected patients carrying homozygous C/C have a higher chance of SVR. In addition, patients who carry T/T (rs8099917) have a higher chance of RVR.

1. Introduction

Infection with the hepatitis C virus (HCV) is a major cause of chronic liver disease, which in turn may lead to the development of cirrhosis and hepatocellular carcinoma (HCC). Cytokines are natural barriers to different viral infections. The human body is capable of producing cytokines differently depending on genetic makeup or polymorphism of the cytokine production genes; this polymorphism affects cytokine production and thus the immune response.

Several antiviral treatments are in the process of authorization. However, the gold standard treatment for chronic HCV infection remains pegylated interferon (PEG-IFN) in combination with ribavirin. Nevertheless, some patients treated with PEG-IFN do not achieve a sustained virological response (SVR) and experience side effects. Moreover it is expensive. Therefore it is important to identify the factors that affect the response to treatment.[1–3]

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Host genetic factors play a vital role in the spontaneous and treatment-induced clearance of HCV infection from infected patients. Recently, genome-wide association studies have identified a single nucleotide polymorphism (SNP) near the IL28B gene on chromosomes 19 that encodes interferon lambda 3 (IFN-λ3),[4] which is considered to be strongly effective against HCV. It is associated with treatment-induced and spontaneous clearance of HCV from infected patients and predicts the response to the antiviral therapy. Several studies have reported that genetic variants of the IL28B gene behave differently to treatment and may be useful to identify a patient's likelihood of response to PEG-IFN treatment.[5–7] Recently Suppiah et al.[8] reported an association between the SNP rs8099917 and SVR. In another study, Tanaka et al. also reported the importance of SNP rs8099917 in terms of treatment response and found the presence of a G allele to be associated with a significantly lower SVR (0% for genotype GG and 78% for genotype TT).[5]

The aim of this study was to investigate the association of SNPs rs8099917 and rs12979860 (IL28B gene) to the response to treatment in HCV genotype 3 patients in Pakistan.

2. Materials and methods

2.1. Patient selection

This study was conducted at the Nuclear Medicine, Oncology and Radiotherapy Institute and Maroof International Hospital from May 2011 to June 2013. The study was approved by the ethics review committees of both hospitals, and patient consent was obtained from all patients enrolled in the study.

One hundred and fifty patients who had been HCV RNA carriers for more than 6 months were screened. Their alanine aminotransferase (ALT) levels had increased to twice the upper limit of the normal range. For inclusion, patients had to have HCV genotype 3, be aged >16 years, male or female, and negative for hepatitis B virus (HBV). Those who fulfilled the study criteria were placed on antiviral therapy. Therapy was started with PEG-IFN alpha-2a 180 μg weekly and 400 mg of ribavirin twice daily. Blood samples from these subjects were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes, which were stored at −80 °C.

2.2. Study design

Patients were evaluated to determine whether they were a responder, non-responder, or sustained responder to treatment. The aim of this study was to assess associations of rs8099917 and rs12979860 genotypes with the response to treatment. A rapid virological response (RVR) was defined as undetectable HCV RNA at the 4th week of treatment. SVR was defined as undetectable HCV RNA at 6 months after the end of treatment. Non-responders were those who had a less than 2 log decline in HCV RNA at week 12 of treatment. Patient relapse was defined as the reappearance of HCV RNA during the follow-up period in patients who had achieved negative HCV RNA at the end of treatment.

Genotyping of all selected serum samples was done using a specific genotyping assay with some modifications.[9,10] The COBAS AmpliPrep/COBAS TaqMan 48 System was used to assess HCV RNA levels (lower limit of detection 15 IU/ml) at baseline, after 4 weeks (RVR) and after 12 weeks of treatment, at the end of treatment (ETR), and at 24 weeks after the end of treatment (SVR).

2.3. Restriction fragment length polymorphism (RFLP) of the amplified product of IL28B

Genomic DNA was extracted from whole blood by phenol/chloroform method. Amplification of 401 bp of the IL28B gene was carried out using primers sense 5′-TTC ACC ATC CTC CTC TCA TCC CTC AT-3′ and antisense 5′-TCC TAA ATT GAC GGG CCA TCT GTT TC-3′ for rs8099917 genotype detection, as reported by Moreira et al.[11] The amplified product was then analyzed on 2% agarose gel. RFLP of the amplified 401-bp product was performed by digestion with BseMI (BsrDI) enzyme to differentiate *IL28B* genotypes of rs8099917 into TT, TG and GG. The restriction pattern of *IL28B* genotype was analyzed on 2% agarose gel. Gel electrophoresis showed bands of different sizes.

For rs12979860 genotype identification, a 694-bp product was amplified using primers sense 5′-AGCAGGACAGATTGGCAAAG-3′ and antisense 5′-CACAATTCCCACCCACCACGAGAC-3′. The amplified product was digested with Hpy166II and analyzed on 2% agarose gel.

2.4. Partial sequencing of the IL28B region

The *IL28B* region was amplified using the sense and antisense primer pairs described above. A 50-μl reaction mixture contained 25 μl of GoTaq Green (Promega), 1.25 pmol of each primer, and 5 μl of extracted DNA. Prior to amplification, desaturation was done at 94 °C for 5 min, followed by 35 cycles of 60 s at 95 °C, 45 s at 58 °C, and 60 s at 72 °C using an ABI GeneAmp 9700 (Applied Biosystems, USA). The amplified product was analyzed on 2% agarose gel using the Gel Doc XR System (Bio-Rad, USA). Fragment sizes were compared with a 100-bp ladder (Fermentas). The product was eluted using the Gene JET Gel Extraction Kit (Fermentas). After purification, these samples were subjected to sequencing using an ABI Automated Sequencer (Applied Biosystems). Chromas version 2.0 was used to analyze nucleotide sequences and electropherograms of each sample. Sequences were aligned using CLC Workbench software (http://www.clcbio.com).

2.5. Statistical analysis

SPSS v. 15.0 software (SPSS Inc., Chicago, IL, USA) was used for the data analysis. Categorical variables are given as the number and percentage. Pearson's Chi-square test was used to estimate the significance of differences between categorical variables. A p-value below 0.05 was considered significant. Univariate and multivariate logistic regression analysis was applied to identify factors with the potential to predict the therapeutic outcome.

3. Results

3.1. Study enrollment and patient characteristics

One hundred and fifty patients were enrolled in the study to determine the correlation of *IL28B* with the response to treatment with a regimen of PEG-IFN alpha plus ribavirin. Twenty patients were excluded from the study due to their refusal to participate. A further 25 patients failed to meet the inclusion criteria. Of the 105 remaining patients, 49 were male and 56 were female. Eligible patients who met the inclusion criteria had detectable HCV RNA in the serum, were positive for HCV antibodies by ELISA, and had HCV genotype 3. All had a platelet count $<180 \times 10^9$/L, white blood cell count $<4.5 \times 10^9$/L, and hemoglobin <12 g/dL. Patients co-infected with HBV or any other viral diseases were excluded from the study. The mean age of the study participants was 41.2 ± 13.5 years. The major mode of acquiring HCV was dental treatment or surgery, although most patients were unaware of the cause of infection. Table 1 summarizes the baseline characteristics of the HCV patients.

3.2. Frequencies of IL28B genotypes in HCV genotype 3 patients

Figures 1 and 2 present the electrophoresis pattern of the restriction of *IL28B* (rs8099917 and rs2979860).