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Roles of ITPA and IL28B genotypes in chronic Hepatitis C patients treated with peginterferon plus ribavirin in Tunisian population

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ABSTRACT

Background: Despite considerable progress in the treatment of chronic hepatitis C, many countries do not have access to these new treatments.
Objectives: Predictive markers of response to treatment are therefore necessary before initiating with historical combination therapy (PEG-IFN + ribavirin) for these populations.
Study design: We therefore evaluated the influence of IL28B polymorphisms on treatment response and Inosine Triphosphate (ITPA) polymorphisms on the incidence of anaemia in a population of 120 Tunisian patients infected with HCV genotype 1b and treated.
Results: The frequencies of favourable IL28B genotypes were 47% (CC for rs12979860) and 63% (TT for rs8099117). Patients in whom favourable IL28B alleles were identified had a higher chance of successful therapy: 82% for CC (rs12979860) and 75% for TT (rs8099117). Viral load decline during the first twelve weeks of treatment was more pronounced in patients with a favourable genotype ($p < 0.0001$). For patients with an unfavourable genotype, the second phase of viral decline was more pronounced in patients with SVR. A viral load decline cut-off of 2.68 log IU/mL at week 12 was best suited to discriminate responders from non-responders with an odds ratio of 40 (95% CI:11.53–170.3). Analysis of ITPA polymorphisms revealed that 16% of Tunisian patients presented ITPase deficiency. None of these patients experienced a decline of ribavirin doses during treatment versus 67% for patients without ITPase deficiency ($p < 0.001$).
Conclusion: These data obtained in a Tunisian population should optimize before and during treatment the chances of success for treatments currently available in Tunisia for chronic HCV infection.

1. Background

Hepatitis C virus (HCV) is responsible for chronic infection in 80% of infected individuals, and more than 100 million persons are currently chronically infected and at increased risk of developing cirrhosis, hepatocellular carcinoma (HCC) and end-stage liver disease [1,2]. HCV infection is therefore a worldwide public health problem [3]. New therapeutic options are now available, but are expensive. The current standard of care in Tunisia is based on a combination of pegylated interferon (PEG-IFN) and ribavirin. However, this therapy achieves sustained virological response (SVR) in only 50% of patients with HCV genotype 1 [4,5].

Genome–wide association studies (GWAS) have revealed that two single nucleotide polymorphisms (SNPs) in the 19q 13 region,

Abbreviations: ITPA, inosine triphosphate; SVR, sustained virological response; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PEG-IFN, pegylated interferon; GWAS, Genome Wide Association Study; SNPs, single nucleotide polymorphisms; III IFN, interferon III; IL28B, interleukine 28 B; HCV RNA, hepatitis C virus ribonucleic acid; RBV, ribavirin; DNA, desoxyribonucleic acid; Hb, haemoglobin; RVR, rapid virological response; W, week; EVR, early virological response; NVR, non virological response; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; BMI, body mass index; ROC, receiver operating characteristic; ISGs, interferon stimulating genes.

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in close proximity to gene IL28B encoding IFN-l (III IFN) family, are highly predictive of a favourable treatment response: rs12979860 [6,7] and rs8099917 [8,9]. Interleukin 28B (IL28B) polymorphisms have been reported to influence SVR following PEG-IFN + ribavirin therapy in HCV-infected patients in the Moroccan [10], Japanese [9,11], Swiss [8], German [12], and Egyptian, Sub-Saharan African and European populations [13].

In addition to IL28B polymorphisms, two frequent variants in the inosine triphosphate (ITPA) gene [14]; rs1127354 and rs7270101, identified in a recent GWAS [15], appear to provide protection against ribavirin-induced haemolytic anaemia in chronic hepatitis C patients [16]. During conventional dual therapy, haemoglobin decreases by approximately 3 g/dL in 50% of patients and by 5 g/dL or more in 10% of patients. These adverse effects of RBV often require dose reduction or treatment discontinuation, leading to decreased efficacy. ITPA polymorphism is related to the haemoglobin decline observed during HCV therapy [15]. The association between inosine triphosphatase (ITPA) genotypes and sustained virological response (SVR) rates in PEG-IFN + ribavirin therapy is controversial [17]. No data are available concerning the influence of IL28B and ITPA polymorphisms on response to HCV therapy and RBV-induced anaemia in the Tunisian population.

2. Objectives

In the present study, we examined the clinical impact of ITPA and IL28B polymorphisms on treatment-induced haematotoxicities and treatment response in Tunisian HCV genotype 1b-infected patients treated with PEG-IFN + ribavirin. As these polymorphisms may modulate treatment responses and treatment pathways, the results of this study could lead to new approaches based on these gene variants for treatment or drug development to achieve safe and effective treatment of Tunisian patients with chronic hepatitis C.

3. Study design

3.1. Patient characteristics

Patients gave their consent to use of genetic material as part of the primary protocol. Official permission was obtained to access the clinical dataset and genetic material. Patients with any other concomitant liver disorders (alcoholic liver disease, autoimmune hepatitis, primary biliary cirrhosis, Wilson disease, HIV infection) or dual infected with hepatitis B were excluded. Patients with treatment adherence of at least 80% were consecutively enrolled the study. All patients were treatment-naïve and infected with HCV genotype 1b. Serum HCV RNA at baseline, treatment weeks 4 and 12, at the end of treatment and at follow-up after treatment were determined by a real-time polymerase chain reaction assay (Real Time HCV; Abbott Molecular). All patients were treated with PEG-IFN (180 μg/week for 2a and 1.5 μg/kg/week for 2b) and ribavirin (RBV) (1200/1000/800 mg/day according to body weight). The clinical characteristics of the study population are described in Table 1. It is a prospective study which started from December 2012 to June 2014. The patients derived from a total of 135 and patients were eliminated in accordance with the following criteria: patients who developed HCC, patients who has stopped their treatment without a doctor permission, patients who have not checked their hemoglobin, ASAT, ALAT for many times or/ and PCR after treatment and patients who have not or lost their medical records for some reasons. DNA and complete clinical datasets were available for 120 patients. During the first week of treatment, all patients received the recommended full dosage of treatment. Haemoglobin levels were determined each week for the first twelve weeks of treatment.

This study included 140 healthy controls who had no medical history of any liver disease, equal number of men/women and were considered as representative of the normal frequencies in the Tunisian population.

3.2. Assessment of efficacy

The endpoint of this study was SVR, defined as negative HCV RNA throughout the 24-week post-treatment follow-up period. Rapid virological response (RVR) was defined as negative HCV RNA at treatment week 4 (W4). Early virological response was defined as negative or at least a 2-log10 decrease from baseline of serum HCV RNA at 12 weeks of treatment. Non-virological response (NVR) was defined as less than a 2-log-unit decline in serum HCV RNA from the pre-treatment baseline value within the first 12 weeks or detectable viraemia 24 weeks after treatment or cases who discontinued treatment due to adverse effects.

3.3. DNA extraction

Peripheral venous blood was collected from all participants in EDTA-containing tubes. For genotyping analysis, genomic DNA was prepared by the extraction method using QIAamp® DNA blood Mini Kit, according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany).

3.4. IL28B and ITPA single nucleotide polymorphisms (SNPs)

Each patient was genotyped for two IL28B SNPs reportedly associated with treatment outcome (rs12979860 and rs8099917) [7]. Genotyping was performed with an ABI TaqMan allelic discrimination kit and the ABI7900HT sequence detection system (both from Applied Biosystems, Courtabeuf, France). Patients were genotyped at polymorphic sites rs1127354, rs7270101 on chromosome 20 using the ABI TaqMan allelic discrimination kit by real-time PCR according to the standard methodology. The possible genotypes for each biallelic polymorphism are as follows: rs1127354: C/C, A/C, A/A (minor allele = A); rs7270101: A/A, A/C, C/C (minor allele = C); rs6051702: A/A, A/C, C/C (minor allele = C). ITPA activity was determined according to the method described by Maeda et al. [18].

3.5. Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Fisher's exact probability test for frequency tables was used for statistical analysis. A chi-square test was used for categorical variables. A receiver operating characteristic curve was used to determine the viral load decrease cut-off between week 0 and 12 that best discriminated between responder patients and non-responder patients. All statistical procedures were performed with R software for Windows.

4. Results

4.1. Patient characteristics

The characteristics of the Tunisian patients included in the study are presented in Table 1. No significant difference was observed between the SVR group (88 patients) and the non-SVR group (32 patients) in terms of FibroTest stage, transaminase levels, baseline creatinine, baseline haemoglobin and baseline viral load. However, age, weight and BMI were significantly lower for SVR patients ($p < 0.001$). Similarly, the type of interferon used influenced the