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Research Article

The Impact of IL28B Single Nucleotide Polymorphism on Antiviral Response in HCV Genotype 2 or 3

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Abstract

Background and Aim: This study aimed to relate the impact of IL-28B SNP in HCV genotype 2 (GT2) and 3 (GT3) associated with the degree of liver fibrosis, according to Metavir score and sustained virologic response (SVR).

Methods: This cross-sectional study conducted between January 2012 and December 2014, which involved 103 patients treated in the center of application and monitoring of injectable drugs from the city of Rio Grande/RS-Brazil. The analysis of the region rs12979860 IL-28B, PCR was performed according to the technique of Moreira et al, 2012.

Results: Caucasian patients showed 3.15 times greater chance of having SVR than the non-Caucasian ones (p = 0.046). Patients with higher degrees of fibrosis (F3/F4) obtain lower rates of SVR. Among the GT3, SVR was 84% in patients with lower degrees of fibrosis (F0/F1/F2) and 67.7% in patients with higher degrees of fibrosis. The GT3 showed fibrosis F3/F4 almost twice more than those with GT2 (59.7% versus 33.3%). Patients with a IL-28B CC genotype had about 3.8 times higher chance to present SVR compared to those patients with IL-28B TT genotype. The chance for advanced fibrosis in patients with TT genotype of the IL-28B was 3.6 times higher than in patients with CC genotype.

Conclusions: The results obtained in this study indicate the need of permanent search for predictive response factors to antiviral treatment of genotype 3 patients, especially those with higher degrees of fibrosis, even in the age of direct-acting antivirals.

Introduction

Although genotypes 2 and 3 have been historically grouped into the same treatment consensus, clinical manifestations such as hepatic steatosis, fast, progression to fibrosis and increased risk of HCC distinguish them, sinceGT3 presents higher incidence of those compared to GT2. [1-5] Current treatment regimens achieve high rates of sustained virologic response in treatment-naïve patients or previously treated without cirrhosis, yet the sustained virologic response in cirrhotic mainly GT3, is lower for unknown reasons. Although systems with sofosbuvir + PEGIFN/RBV show higher rates of adverse effects and require increased monitoring, the short-term (12 weeks) of this treatment associated with superior results, makes this regimen recommended to IFN-eligible patients; until other therapeutic options free of it demonstrate superior results.

The response to treatment depends on viral factors such as genotype and viral load; and host genetic factors [6-8]. Among the factors related to the host, the single nucleotide polymorphisms (SNP), the rs12979860 and rs8099917 in close proximity to the IL-28B are highlighted [9-11]. Belonging to the type III interferon family, the Interleukin-28B (IL-28B) is a chemical messenger of immune reactions and it presents antiviral activity, besides being related to Interferon (IFN) alpha [10,12]. The IL-28B was discovered in 2003 by two independent groups, through the identification of structural differences in the type I IFN [13,14]. Although the main role of both groups of interferons is related to antiviral activity, the INFλ has aroused much interest due to its relation to spontaneous resolution and successful treatment of HCV infection [15].

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Therefore, given the diversity concerning HCV infection, this study aims to measure the impact of IL-28B SNP on HCV genotype 3 associated with the degree of hepatic fibrosis and sustained virologic response.

Materials and Methods

This is a cross-sectional study, which was conducted between January 2012 and December 2014. It was approved by the Ethics Committee of the Federal University of Rio Grande according to resolution 145/2014 (Annex 1). All patients signed an informed consent form (Annex 2) and answered a questionnaire (Appendix 3) for the purpose of socio-demographic and behavioral data. Biochemical variables were obtained from searches to medical records.

Treatment

All patients with chronic HCV were treated with subcutaneous injections of conventional interferon (IFN) or Pegylated (PEGIFN), plus oral dose of Ribavirin (RBV) adjusted according to their weight.

Samples and Laboratory Techniques

The next step was the brushing sampling collect, which were placed into cryogenic tubes (4.5 ml) containing Tris-EDTA buffer and screw cap. The samples were sent to the Molecular Biology Laboratory of Interdisciplinary Area in Biomedical Sciences, Medical School - FURG. The oral brushing samples were frozen at -20° C. DNA extraction was performed by the use of the Invitrogen® commercial kit following the extraction protocol for epithelial cells. The quality control for assurance of genomic DNA extraction was performed using polymerase chain reaction (PCR) for CCR2 gene. Analysis ofIL-28 rs12979860 of was performed by PCR and sequencing according to IL28B-860F primers: AGCAGGACAGATTGGCAAAG; IL28B-860R: CACAATTCCCACCACGAGAC, in compliance with the technique by Moreira et al [16].

The reaction was performed in a final volume of 50 µLcontaining: 50-500 ng of template DNA; 1 U of Platinum Taq DNA polymerase (Invitrogen, São Paulo, Brazil); 200 M of each deoxynucleoside triphosphate (GE Healthcare, Little Chalfont, UK); 1.5 mM MgCl2 (Invitrogen®); PCR buffer (20 mMTris-HCl, pH 8.4, 50 mMKCl) and 10 pmol of each primer (Eurofins MWG Operon, Huntsville, USA). The cycling conditions were: 94 ° C for 3 minutes followed by 40 cycles of 94 ° C for 30 seconds, 60 ° C for 30 seconds, 72 ° for 1 minute and 7 min at 72 ° to the final length. It was used 1% agarose gel to visualize 694pb PCR products.

Variables

Variables obtained from medical records: HCV genotype, fibrosis stage (Metavir score), viral load, platelets, Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST). Variables such as: age, gender, education level, race, marital status, blood transfusion, health care professional, injecting drug use, inhaled cocaine use, syringe sharing, surgical procedures, tattoos or piercing and Sexually Transmitted Diseases (STDs) were analyzed by applying the questionnaire.

Statistical analysis

Initially, the demographic, clinical and laboratory variables were described as well as the risk factors for hepatitis C. The distribution of continuous variables was assessed through histogram and the asymmetrical variables were converted into natural logarithm for subsequent exponential analysis. The logarithm average from ALT and AST before and after treatment was compared by the paired t-test. The ratio of HCV genotype and IL-28B genotype with RVS and the degree of fibrosis was assessed through the use of logistic regression. The outcomes used were RVS and degree of fibrosis. All the analyses were performed in Stata 14.1(Statcorp, College Station, TX, USA). As a cutoff point the value $p<0.05$ was used for statistical significance.

Results

Throughout the study266 patients started antiviral treatment for HCV, 103 of them were infected with HCV (genotypes 2 and 3) and were assisted at the Center of Application and Monitoring of Injectable drugs (CAMMI) in the city of Rio Grande/RS-Brazil. One of the patients refused to participate in the study. Two patients died before the end of the research and two other were not found neither by telephone nor home visits.

Among 98 evaluated patients, the majority 55 (56%) were male, Caucasian 81 (83%). The average age was 55.8 years (SD ± 9.9), ranging from 29 to 71 years. The most frequent IL-28B genotype was CT 43 (46%), followed by CC 30 (32%). Among the analyzed HCV genotypes the GT3 was most prevalent (84%). In this study, 34 (35%) of the patients were in retreatment. Approximately 70% patients underwent a biopsy, 41 of them (55%) presented degree of fibrosis 3 or 4.

The clinical laboratory profile of patients is described in (Table 1). Only 1% and 2% were positive for anti-HBc and anti-HIV, respectively. Almost all patients (97%) were treated with PEGIFN and RBV. The SVR was achieved in 74 (76%) patients.

Table 1: Laboratory profile of patients with hepatitis C evaluated (n = 98) at CAMMI in Rio Grande, RS.

	N	%
Current Treatment Regimen		
PEGIFN and RBV	95	96.9
Convencional INF	3	3.1
PreviousTreatment Regimen		
Had no treatment before	66	67.4
PEGIFN and RBV	25	25.5
Conventional IFN	7	7.1
Treatment Final Viral Load		
Undetectable (<12UI/ml)	85	89.5
Detectable (>12UI/ml)	10	10.5
SVR		
Responder (<12UI/ml)	74	76.3
Non responder (>12UI/ml)	23	23.7

PEGIFN: Pegylated Interferon, RBV: Ribavirin, SVR: Sustained Virologic Response.

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Table 2: Median and inter quartile range of the results of aminotransferase before and after treatment in patients with hepatitis C (n = 98) in CAMMI of Rio Grande, RS.

	Before treatment		After Treatment	
	Median	IQR	Median	IQR
Male				
AST	51	30-93	32	21-50
ALT	62,5	40-109	32	18-55
Female				
AST	70	36-110.5	30	23-41
ALT	79,5	38-136	28	20-38

IQR: interquartile range, AST: aspartateaminotransferase, ALT: alanineaminotransferase.

Regarding risk factors for hepatitis C among the evaluated patients the most often reported were undergone surgery (84%), not using condom with regular partners (71%) and casual partners (64%), blood transfusion (33%), use of injecting drugs (22%), needle and syringe sharing (21%) and presence of tattoo and / or piercing and / or acupuncture procedure (16%). None reported being hemophiliac and one patient was on hemodialysis. About 14% of patients had two or more sexual partners over the last year. Concerning sexual orientation, only one reported being homosexual/bisexual.

Considering the average of enzymes (Table 2) ALT and AST before and after treatment it is observed a decrease in the value of both tests after treatment in both sexes ($p < 0.001$). However, the number of platelets before (158.86 ± 62.27) and after treatment (154.08 ± 65.97) did not change ($p = 0.258$).

Table 3: Relation of gender, color, degree of fibrosis and HCV IL-28B genotypes with SVR in patients with hepatitis C (n = 98) at CAMMI in Rio Grande, RS.

	Prevalence (%)	OR (CI95%)	p-value¥
Gender			0.158
Male	70.9	2.05 (0.75; 5.57)	
Female	83.3	1	
Skin Color			0.046
Caucasian	80.3	3.15 (1.02; 9.77)	
Non Caucasian	56.2	1	
Degreeoffibrose			0.093
F0/F1/F2	84.8	2.70 (0.85; 8.59)	
F3/F4	67.5	1	
HCV Genotype			0.709
GT2	80	1.29 (0.33; 5.04)	
GT3	75.6	1	
IL-28BGenotype			0.137
CC	86.7	3.79 (0.93; 15.5)	
CT	72.1	1.51 (0.48; 4.74)	
TT	63.1	1	

¥ Wald test obtained using logistic regression.

Table 4: Relation of gender, skin color, HCV genotype and IL-28B with fibrosis F3/F4 in patients with hepatitis C (n = 98) at CAMMI in Rio Grande, RS.

	Prevalence (%)	OR (CI95%)	p-value¥
Gender			0.731
Male	57.1	1,18 (0,47; 2,97)	
Female	53.1	1	
Skin Color			0.689
Caucasian	54.2	1	
Non Caucasian	60	1.27 (0.40; 4.01)	
HCV Genotype			0.185
GT2	33.3	1	
GT3	59.7	2.96 (0.80; 10.89)	
IL-28BGenotype			0.185
CC	43.5	1	
CT	55.9	1.65 (0.57; 4.78)	
TT	73.3	3.57 (0.87; 14.65)	

¥ Wald test obtained using logistic regression.

About the relation between HCV genotypes and IL-28B with the SVR, table 3, it is observed that the prevalence of SVR was similar among HCV genotypes, around 80%. Patients with IL-28B CC genotype showed about 3.8 times higher chance of SVR compared to those patients with IL-28B TT genotype; however the confidence interval includes 1 unit. From all the patients without SVR, 17.4% were CC genotype, 52.2% and 30.4% were TT, while those with SVR, 37.7% were CC genotype, 44.9% CT and 17.4% TT ($p = 0.150$).

The relation between HCV genotypes and IL-28B with the occurrence of fibrosis (F3/F4) is shown in (Table 4). The prevalence of advanced fibrosis was 2.96 times higher in patients with HCV genotype 3 than those with genotype 2. Patients with IL-28B TT genotype showed 3.6 times higher chance of presenting advanced fibrosis than patients with CC genotype, but the difference did not reach statistical significance.

It is observed that the prevalence of SVR in fibrosis F3/F4 was similar among genders and among HCV genotypes, about 80%. According to the results, Caucasian patients showed 3.15 times more chance of having SVR than the non-Caucasian ($p = 0.046$). The patients who presented degree of fibrosis F0/F1/F2 obtained more SVR than those with a degree of fibrosis F3/F4. Patients with a IL-28B CC geno type had about 3.8 times more chance for SVR compared to those patients with IL-28B TT genotype; however the confidence interval included 1unit.

Patients with HCV GT3 evidenced a prevalence of fibrosis (F3/F4) almost twice higher than those with GT2 (59.7% versus 33.3%). The chance of having advanced fibrosis in patients with IL-28B TT genotype was 3.6 times higher than in patients with CC genotype, but the difference did not reach statistical significance.

The prevalence of SVR in patients which degrees of fibrosis ranged from F0/F1/F2 and F3/F4 was similar in HCV GT2 and GT3. The prevalence of SVR in patients with a degree of fibrosis F3/F4 was 66.7% for G2 and 67.6% forG3 patients ($p = 0974$). When comparing the different degrees of fibrosis, the difference was also not evident ($p = 0.425$ for G2 and $p = 0.147$ for G3).

Discussion

This research was carried out at the reference center for treatment of HCV in southern Brazil and it has mapped the sociodemographic, clinical laboratory and immunogenetic profile of patients infected with HCV. Viral factors such as genotype and viral load, and host factors such as gender, age and ethnicity contribute to progression of hepatic fibrosis and also to the antiviral therapy among infected individuals. In recent years, analysis of host SNP has become a research target in an attempt to find the predictors of sustained virologic response for treatment. The relation between polymorphism in the gene which promotes IL-28B and the outcome and response to treatment of HCV has been discussed; however studies have reported conflicting results [17,18].

In Brazil, genotype 1 (65%) and 3 (30%) are the most prevalent, followed by 2 (5%). The highest prevalence of GT3 is in the South (43.2%) and GT2 in the Midwest (11.4%).19In this study, from all patients treated at CAMMI, 57.0% were genotype 1 (GT1) and 42.9% GT2 and GT3, being 83.7% of them GT3, which is equivalent to 35.9% in total of patients assisted during this period.

The analysis of IL-28B polymorphisms among the patients who participated in the study showed that the CT genotype (46%) is more common among individuals with HCV followed by CC (32%) and TT (22%); disagreeing with other studies, as ESLAM et al [20]. which demonstrates the CC polymorphism (46.9%) as the most prevalent in the population studied. In contrast, [21] corroborate with our study, since it found 48.3% CT in patients infected with HCV.

Two meta-analyzes that analyzed the role of IL-28B in GT2 and GT3 have been recently published [22,23] and they also found conflicting results as showed in individual studies [17,18]. In the study held by Chen et al, [22], IL-28B rs12979860 CC was a strong predictor of SVR in GT1 patients treated with PEGIFN, regardless of their ethnicity. When HCV GT2 and GT3 were analyzed, this polymorphism was not statistically considered a predictive factor of SVR.

In the present study, patients with IL-28B CC genotype had about 3.8 times higher chance of SVR compared to those patients with TT genotype, however the confidence interval includes 1 unit. Although the lack of statistical significance, Caucasian patients presented OR 3.16 (1.02, 9.77) when associated with SVR, according to literature in several studies [23,24].

The study cited above, by Rangnerkar and Fontana [23] found that the favorable genotypeIL-28B (CC) is a predictor of SVR and rapid virologic response (RVR) in GT3 Caucasian patients; in GT2 Asian patients, the favorable IL-28B genotype was associated with RVR, but not with SVR, both treated with PEGIFN and RBV for 24 weeks. This study includes patients who were treated and clinically assisted according to the Brazilian PCDT, which does not include request of the viral load in the 4th week after starting treatment - RVR, due to this limitation we have no data concerning RVR to compare with other studies.

Due to the high rates of SVR in patients infected with GT2, predictors of response are not very useful; and its use is limited to identifying patients who may have reduced treatment duration. 25This study found around 80% of SVR in GT2; in contrast, in patients infected with GT3, with advanced degrees of fibrosis the SVR rates are unsatisfactory even in IFN-free regimens, what induces a continuous search for predictors of response [26].

In the present study, the SVR in GT3was around 75.6%, including all degrees of fibrosis; however when higher degrees of fibrosis were evaluated separately, the SVR was 67.6%. These data affirm the influence of advanced degrees of fibrosis in response to antiviral treatment, corroborating the findings of research by Poynard and colleagues [27].

Variants of IL-28B have been described as having a significant effect on progression of fibrosis in patients infected with HCV [28,29]. According to expectations, when the degree of fibrosis and IL-28 polymorphism were analyzed, TT genotype was associated with higher advanced degrees of fibrosis (73.3%), while CC was 43.5% [25,21].

Considering the high cost of antiviral therapy, especially with the advent of DAAs, which there is still no knowledge on its effect over time, particularly in free interferon regimen [30,31] and especially the possible resistance mechanisms, the determination of new genetic markers becomes fundamental in treatment decisions for patients with chronic hepatitis C.

Conclusion

The results of this study contribute to the establishment of possible genetic markers for response to treatment of hepatitis C. This knowledge brings a prognostic significance in chronically infected patients, besides it enables direct the therapy more effectively at lower cost.

Considering that the HCV infection involves several factors related to the host and many other relevant factors to the virus, it is justified the controversial and conflicting results concerning IL-28B polymorphisms and sustained virologic response. It is important to highlight that the IL-28B CC genotype is associated with double response to antiviral treatment in relation to TT, as well as higher chance of spontaneous healing, regardless of ethnicity; being found mostly in Caucasians [32].

Whereas HCV GT3, with higher degrees of fibrosis (F3/F4) shows lower rates of SVR, even in treatment regimens with DAAs, it requires continuous search for predictors of response for anti viral therapy. It is hoped that this study will further contribute for clarification on the factors regarding the host and its implications on therapeutic response.

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