INVITED LECTURES

IL02 POLYMORPHISM IL28B AND RESPONSE TO THERAPY IN CHRONIC HEPATITIS C

Genadieva-Dimitrova M, Calovska-Ivanova V, JoksimovicN, Trajkovska M, Todorovska B, Popova R, Miloshevski M, Curakova E, Stardelova K, Andreevski V, Janevska D
University Clinic of Gastroenterohepatology, Skopje, Medical Faculty Skopje, Macedonia

INTRODUCTION: Chronic hepatitis C is still a majgor cause for developing cirrhosis and hepatocellular carcinoma which often results in liver failure and thus in liver transplantation. According to the World Health Organisation 180 million people are infected worldwide and 3-4 million new infections per year were estimated (1). The current standard of care (SOC) for chronic HCV infection is a combination of pegylated interferon (PegINF α-2a or PegINF α-2b) plus body-weighted ribavirin (RBV) for the duration of 24 weeks or 48 weeks depending on the HCV viral genotypes (2). The primary goal of the treatment is HCV eradication, which is actally sustained viral response (SVR). The SVR is defined as undetectable HCV RNA in serum, 24 weeks after the completion of the antiviral treatment (3). However, only about 40-50% genotype 1 or 4 patients treated and 80% genotype 2 or 3 patients treated could respond completely and achive sustained virological response (4,5). Moreover, side effects from the therapy such as influenza-like symptoms, psychiatric symptoms and hematological abnormalities, could result in the dose reduction or even the premature discontinuation of the treatment (6). To avoid these potential adverse events in patients who do not benefit from the treatment and to reduce the cost of therapy, it is necessary to predict an individual's response before at the early stage of the treatment. Virus-specific characteristic (viral load, genotype, viral variants as mutations of interferon sensitivity determining region- ISDR) may be responsible for virologic response but also clinical parametars (age, gender, BMI, fibrosis stage, liver enzymes) (7,8). Investigations on genetic determinants of chronic hepatis C established that a single nucleotide polymorphism (SNP) in the interleukin (IL)-28B gene promoter region affected the spontaneous and induced clearance of hepatitis C virus (9). Among 500 000 genetic variants which were analyzed genome-wide, a few associated with virologic response were identified, and showed variable frequency and importance across human ethnic groups (10). The mechanism by which SNPs influence the outcome of HCV infection and its treatment is not clear. It is suggested that regulation of the promoter region of IL28B in antiviral activity may also affect two other genes belonging to interferon (INF)-λ family encoded in this region (10, 11). INF-λ possess antiviral activities agains hepatitis C virus (12). The genome-wide associated studies (GWAS) showed that SNPs near IL28B gene (CC for rs12979860, TT for rs8099917 and AA for rs12980275) were associated significantly with treatment outcome in patient with chronic hepatitis C. However, about 50% of patients with a sustained virological response do not carry favorable IL28B alleles (13). The factors which increase the chance of a therapeutic response in these patient are not yet known. A detailed analysis if the course of therapy of chronic hepatitis C with pegylated INFα and ribavirin in the presence of a hazardous IL28B allele might better delineate the clinical

characteristics of the difficult-to-treat group of patients. The aim of the study was to evaluate the effects of IL28B polymorphism on response to treatment with peginterferon and ribavirin in patients with chronic hepatitis C.

MATERIAL AND METHODS: Twenty-five adult Caucasians previous assessed with chronic hepatitis C due to HCV genotupe 1 and 3 were included in the study. The study protocol was approved by the institutional ethics committee and written informed consent was obtained from each study participant. Patients were treated with standard antiviral therapy with pegylated INF alfa and ribavirin. Pegylated interferon alfa 2a 180 μg was administered subcutaneously once a week. Body-weighted ribavirin was administered daily. The treatment duration was 48 weeks for patients with HCV genotype 1 and 24 weeks for patients infected with HCV genotype 3. SVR was used for the assessment of the antiviral treatment effectiveness. SVR is defined by undetectable viral RNA 24 weeks after the end of treatment. Finaly eighteen patients were analysed, because two premature discontinuated the treatment and for five there are no available data for SVR. Sample od peripheral blood were collected from each patient enrolled in the study for HCV quatntification and IL28B polymorphism genotyping. Reverse transcriptase-polymerase chain reaction assay for HCV quantification was done with One-tube real time PCR HCV amplification with lower detection limit 70 IU/ml. Polymorphisms rs12979860 (C>T) and rs8099917 (T>G) in gene IL28B were genotyed by PCR. Each polymorphism assay contained one pair of primers and one pair of probes, and each allele of the polymorphisms was labeled. For statistical analysis mean and standard deviation were used for parametric variables. Considering the small sample difference test (percentage of structure) was used to determinate the genetic predictors of the SVR. A $p<0.05$ was consideded statistically significant.

RESULTS: The study population included 9 genotype 1 and 9 genotype 3 HCV infected patients. Their median age was 31.4±4.4 years and 88.88% were males. SVR were achived in 83.3% patients. The distribution of the frequencies od rs12979860 genotypes in the analyzed sample was: 10 (55.55%) patients with CC genotype and 8 (44.44%) patients with CT genotype. The distribution of the frequencies od rs8099917 genotypes was: 15 (83.33%) patients with TT genotype and 3 (16.66%) patients with TG genotype. The difference test showed that difference in persentage which is registered between SVR in CC and CT is not statistically significant (p=0,6714). There is no association in achievement SVR in CC and non-CC genotypes of rs12979860. There also no significance in achieving SVR in patients with TT genotype of rs8099917 and in patients with TG genotypes (p=0.3961). Futhermore there was no association with the achivment od SVR as compared with genotype (p=0.0579).

DISCUSION: In this study, no significant difference was found in the response to treatment and allele proportions of SNPs rs12979860 and rs8099917 possibly due to the small size of the sample. The study of Silva Conde et al. confirmed similar effect of IL28B polymorphism on SVR in infected HCV patients (14). In another study from Norway and Denmark involving genotype 3 HCV-infected patients, RVR was achived by a significantly greater number of

patients who had CC and TT genotypes at rs 12979860 and rs8099917, respectively, but these genotypes showed no association with SVR (15). Consistent to our findings were results of the study of Sarrazin et al.which present no significant association of SNPa rs8099917 with virologic treatment response (16). Investigation of a comparable number of genotype 2/3 infected patients in study by Rauch et al. also showed no correlation between the rs8099917 genotype and virologic response to pegylated interferon/ribavirin combination therapy (17). In contrast, the GWAS identified that homozygosis for C allele of rs12979860 and homozygosis for the T allele of rs8099917 were favorable genotypes of the IL28B gene polymorphisms which predicted the SVR in patients with chronic hepatitis C treated with peginterferon and ribavirin (18,19). The distribution of frequencies of rs12979860 genotypes in our study group was : CC 55.55% and CT in 44.44% . The distribution of frequencies of rs8099917 genotypes in our study sample was TT 83.33% and TG 16.66%. Sticchi et al. reported distribution of rs8099917 genotypes TT in 55%, TG in 40% and GG in 5% of the study participants (20). Results of other studies reported bigger percentage of CT than CC for distribution of the frequencies of rs12979860 (21.22). However, about 50% of patients with a sustained virological response do not carry favorable IL28B alleles (13). The factors which increase the chance of a therapeutic response in these patient are not yet known. Other factors, such as HCV genotype, viral-load, ethnicity should be used together with IL28B genotype as predictors of response on antiviral therapy.

CONCLUSION: We did not find a significant association of SNPs rs12979860 and rs8099917 with SVR thus disagreeing with studies that found an association between genotype CC (rs12979860) and SVR in individuals with genotype 1, 2 and 3 as well as between genotype TT (rs 8099917) and SVR in individuals with genotype 3. Our study is limited by its sample size. And possibly results due to this fact. Nevertheless genotyping of this polymorphism on a large HCV population will aid clinical decision making for both current standard care and potentially for the integration of other agents in future, providing an opportunity for clinicians to individualize treatment regimens for hepatitis C patients.

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