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SNP RS12979860 RELATED SPONTANEOUS CLEARANCE OF HEPATITIS C VIRUS INFECTION IN HCV/HIV-1 COINFECTED PATIENTS

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ABSTRACT

CC genotype of SNP rs12979860 promotes spontaneous HCV clearance in monoinfected patients.
THE AIM of this analysis was evaluation of impact of rs12979860 polymorphism on HIV or HCV viral load, CD3, CD4 and CD8 count as well as HCV clearance among HCV/HIV coinfected patients.
MATERIALS AND METHODS. The study included 41 consecutive HCV/HIV coinfected patients. HIV RNA, HCV RNA, HCV genotype and rs12979860 polymorphism sequence as well as CD3, CD4 and CD8 cells count were analyzed in all patients.
RESULTS. CC genotype rs12979860 was identified in 16 from 41 patients. During at least 4 years follow-up, five genotype CC patients (31%) became HCV RNA undetectable, that was not a case in CT and TT patients. No statistical differences in HIV viral load and the number of CD3, CD4 and CD8 related to rs12979860 polymorphism were observed. The baseline level of HCV RNA in patients with CC genotype was significantly lower compared to patients with non-CC genotypes (88546 ±74181 vs. 726021 ±30709 IU/mL).
CONCLUSION. CC genotype related to SNP rs12979860 can affect the lower level of HCV viral load compared to patients with CT and TT genotypes and promotes spontaneous clearance of HCV RNA in HCV/HIV coinfected patients.

Keywords: *HIV/HCV coinfection, SNP rs12979860, HCV clearance*

INTRODUCTION

HCV infection is estimated to affect 2.4% of the worldwide population (1, 2). One of the most important issue in era of new anti-HCV agents is prediction of virologic response or HCV spontaneous clearance based on baseline or on treatment evidences (3). Frequent spontaneous elimination of HCV is associated with single nucleotyde polymorphism (SNP) at site rs12979860 of chromosome 19. HCV monoinfected patients with CC genotype demonstrate spontaneous HCV clearance more often than those with genotype CT or TT (4). Higher efficacy of treatment with pegylated interferon alfa (PegIFNα) and ribavirin (RBV) was demonstrated in both HCV monoinfected and HIV/HCV coinfected genotype CC patients compared to non-CC patients (5).

The aim of this analysis was evaluation of SNP rs12979860 polymorphism on spontaneous HCV clearance, HCV and HIV viral load, as well as CD3, CD4 and CD8 count in HCV/HIV coinfected patients.

MATERIALS AND METHODS

The study included 41 consecutive patients (10 females and 31 males) aged from 27 to 50 years who were diagnosed as infected with HIV and positive for anti-HCV. Patients who did not received anti-HCV or ART (Antiretroviral Therapy) treatment were followed for at least 4 (mean: 5,6 ±0,22) years. HIV RNA and HCV RNA concentrations were measured, HCV genotype was determined as well as lymphocytes CD3, CD4 and CD8 counts were assessed at baseline.

Diagnosis of HIV infection was based on detection the HIV antibodies with ELISA and Western blot testing (Cambridge Biotech Corporation, USA). HIV viral load was determined by RT-PCR using the Cobas Amplicor HIS 1.5 (Ultra-Sensitive). Subpopulations of CD3, CD4

and CD8 in blood were analized by flow cytometry using Becton Dickinson 2 instrument. HCV-RNA level and its genotype were determinated with RT-nested-PCR (Syngen Biotech, USA), with sensitivity of 25 IU/mL. Rs12979860 polimorphism sequence detection was carried out by direct PCR product sequencing. DNA was isolated from the peripherial blood using the reagent set and automatic device for nucleic acid isolation EasyMag (Biomereueix, France) according to the manufacturer's protocol. Briefly, 50 µl of whole blood was mixed with 500 µl of the Lysis Buffer (Biomereueix, France) and incubated at 37^0C for 1 h with 30 µl of Proteinaza K solution (10 mg/ml, Sigma). Proteolysis was followed by DNA binding to the added magnetic beads (Silica) ((Biomereueix, France), washing and eluting into the 50 µl of the Washing Buffer 3 ((Biomereueix, France). All the steps of proteolyzed blood processing were performed automatically in EasyMag machine. A portion of IL28B gene flanking the polimorphic site of interest was amplified in PCR reaction in 20 µl of reaction mixture containing 1xPCR buffer, 200 µM dNTPs, 0,5 µM each of sense and antysense primers, 1 U TaqRed DNA Polymerase (EURx, Poland, Gdansk) and about 50 ng of DNA. The primer sequence was as follows: sense 5'-GCTTATCGCATACGGCTAGG-3', antisense 5'-AGGCTCAGGGTCAATCACAG-3'. Cycle conditions for PCR reaction were as follows: after initial hold for 5 minutes at 95^0C, the samples were cycled 40 times at 95^0C for 15 seconds, 60^0C for 1 minute and 72^0C for sec, followed by the terminal PCR products extension for 5 min at 72^0C. PCR products were analyzed on agarose gel, excited from the gel and purified with the "Gel-Out" set of reagents (A&A Biotechnology, Poland, Gdansk). PCR products sequencing were performed with "Big Dye Terminator v3.1 Sequencing Standard Kit" and 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA).

The study was approved by the Ethical Committee of Medical University in Białystok. Informed consent including approval for genetic studies was obtained from each patient.

Statistical analysis was performed with Mann-Whitney test. Values of $p<0.05$ were considered as statistically significant. The Statistics 10.0 for Windows was applied to carry out the analysis (Statsoft Inc., Tulsa, USA).

RESULTS

CC genotype was identified in 16 patients, while in 20 CT and in 5 TT rs12979860 polymorphism was observed. Twenty-two patients were infected with genotype 1 HCV, seven with genotype 4, and seven with genotype 3. During follow-up period HCV-RNA became undetectable in five genotype CC patients (31%). Among the five patients who cleared HCV RNA, genotype was not specified. In contrast HCV RNA clearance was not observed in any non-CC patients. Baseline HCV RNA level in CC patients (88546 ±74181 IU/mL) was significantly lower than in non-CC patients (726021 ±30709 IU/mL, p=0.048). As shown in table I, mean HIV RNA was more than ten times lower in CC compared to non-CC patients, but the difference was not statistically significant (32073 ±9165 vs. 52735 ±17730 IU/mL) (Fig1). There were no significant differences between CC versus non-CC patients regarding CD3, CD4 and CD8 count (Tab. 1).

Table I. Mean (±SE) BMI, HIV RNA, HCV RNA and CD3, CD4 and CD8 lymphocytes subsets in patients with CC versus non-CC (CT or TT) genotypes.

Parameter	SNP rs12979860 genotypes		p
	CC (n=13)	non-CC (n=18)	
BMI	21.9 ±2.9	22.1 ±2.6	0.836
HIV RNA, (IU/mL)	32073 ±9165	52735 ±17730	0.774
HCV RNA (IU/mL)	88546 ±74181	726021 ±30709	**0.046**
CD3 (µl)	1010 ±151	1161 ±194	0.902
CD4 (µl)	308 ±67	285 ±46	0.774
CD8 (µl)	850 ±250	823 ±145	0.902

DISCUSSION

The first publications demonstrating beneficial effects of CC genotype of rs8099917 polymorphism in spontaneous clearance of hepatitis C virus and efficacy of HCV genotype 1 treatment in patients with HIV/HCV coinfecion was published in 2010 (6). However publications on spontaneous clearance of HCV infection among HCV/HIV infected patients are sporadic. *Sajadi* et al. (7) pointed out lack of association between HIV viral load and rs 12979860 genotypes and considered possibility of spontaneous HCV clearances among HCV/HIV co-infected patients, as related to CC genotype. Results in this paper are in line with these observations. We demonstrated nine times lower HCV viral load in CC patients compared to non-CC, that suggest possible trend to viral clearence. Therefore we can assume that CC genotype of rs12979860 polymorphism may be an useful baseline predictor of HCV clearance in HCV/HIV coinfected patients (8). However to confirm this concept prospective studies should be carried-out in this population. *Lunge* et al. (9) observed spontaneous clearances of HCV in 24.6% Brazilians living with HIV/HCV, that was significantly more frequent in genotype CC patients. *Clausen* et al. (10) described for the first time in europeans frequent spontaneous HCV clearances in HCV/HIV co-infected patients with CC genotypes (23%).

CONCLUSION

The CC genotype of rs12979860 polymorphism may affect the HCV viral load lower compared to patients of the CT and TT genotypes, and is conducive to a spontaneous elimination of HCV infection in patients with HIV / HCV coinfected. Rs12979860 polymorphisms is not associated with BMI, HIV viral load and lymphocytes subpopulations count.

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