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STUDY OF THE HEPATITIS C VIRUS IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Hepatitis C virus (HCV) is a major public health problem. It is a leading cause of chronic liver disease and the most common indication for liver transplantation. The therapy for eradication of HCV infection is successful in only 50.0-80.0% of patients and is highly dependent on the HCV genotype.

Molecular detection and characterization of HCV in the Republic of Macedonia started in 1990. Since then, more than 4000 samples have been analyzed at the Research Centre for Genetic Engineering and Biotechnology (RCGEB) "Georgi D. Efremov," Skopje, Republic of Macedonia. The prevalence of HCV infections in the healthy population of the Republic of Macedonia was found to be 0.4%, while it varies between 23.0 and 43.0% in different at-risk groups of patients.

The prevalence of HCV genotypes, according to associated risk factors in HCV infected patients from the Republic of Macedonia, was analyzed. We found genotype 1 to be predominant in a group of hemodialysis patients, while genotype 3 was predominant in intravenous (IV) drug users.

Association of six polymorphisms in the Oligoadenylate synthetase (*OASL*)-like interferon-stimulated gene with a sustained virological response was also analyzed. Our preliminary results suggest that non ancestral alleles in four of the six studies polymorphisms in *OASL* gene are associated with sustained virological response among HCV infected patients in R. Macedonia.

Keywords: Hepatitis C virus (HCV) Genotyping, Intravenous (IV) drug users, HCV therapy, Oligoadenylate synthetase (*OASL*) like interferon stimulated gene

INTRODUCTION

Hepatitis C virus (HCV) is a major health problem affecting 170 million people worldwide. The prevalence rate is about 1.0% in western countries and North America, 3.0-4.0% in some Mediterranean and Asian countries and up to 10.0-20.0% in parts of central Africa [1]. The HCV infection is the leading cause of chronic hepatitis worldwide, progressing to liver cirrhosis, and hepatocellular carcinoma in approximately 20.0% of patients [2-5].

Hepatitis C virus was the first virus discovered by molecular cloning [6]. Hepatitis C virus is an enveloped, positive-sense RNA virus, belonging to the Hepacivirus genus of the Flaviviridae family [6]. The genome is approximately 9.6 kb in size

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and contains an open reading frame (ORF) encoding a large protein precursor [7]. This precursor is cleaved by host and viral proteases into various structural and non structural proteins. The structural proteins are at the 5' end and include the capsid or core protein (c), two envelope proteins (E1 and E2) and a small protein of unknown function (P7). The structural proteins are followed by at least six non structural (NS) proteins denoted as NS2, NS3, NS4A, NS4B, NS5A and NS5B [8].

On the basis of phylogenetic analysis of nucleotide sequences, HCV can be divided into six genotypes and several subtypes. The six genotypes differ between each other in 30.0-35.0% of sequence, while the subtypes differ in 20.0-25.0% over the complete genome. Different HCV genotypes exhibit different epidemiological and clinical implications. The HCV genotypes 1, 2, and 3 appear to have a worldwide distribution. The HCV genotypes 1 and 3 are the most common genotypes in the United States [9] and in Europe [10,11]. Genotype 3, which is endemic in Southeast Asia, has been encountered in Europe and the USA, with relatively high frequency in intravenous (IV) drug users [12]. The HCV genotype 4 appears to be prevalent in North Africa and the Middle East [13,14], and genotype 5 predominates in South Africa. Genotype 6, according to different authors, is divided into additional genotypes 7-11 and is mainly found in the HCV populations of Vietnam and Hong Kong [15-19]. Hepatitis C virus genotypes have proved to be important epidemiologic marker that can be used to predict success of therapy. The HCV genotype 1 is a more aggressive strain and one that is less likely to respond to interferon treatment than HCV genotypes 2 or 3. All this information has great significance when planning future strategies for eradication and therapeutic management of HCV.

Molecular detection and characterization of the HCV infections in the Republic of Macedonia started in 1990. Since then, more than 4000 samples have been analyzed at the Research Centre for Genetic Engineering and Biotechnology (RCGEB) "Georgi D. Efremov," Skopje, Republic of Macedonia. Blood samples were collected by the Clinic of Infection Diseases, Clinic of Gastroenterology and several dialysis centers from the Republic of Macedonia. Amplicor Specimen Preparation and Amplification kit (Roche Diagnostics, Indianapolis, IN, USA) were used for RNA isolation and amplification according to the manufacturer's recommendations. The prevalence of HCV infections in the healthy population of the Republic of Macedonia was found to be 0.4%, while in different at-risk groups of patients such as IV drug users, hemodialysis patients, patients under a blood transfusion regimen and those with unknown factors, the prevalence of HCV varies between 23.0 and 43.0% [20].

Genotyping analyses were performed on 1346 patients with a positive HCV-RNA analysis result. The HCV/RNA genotyping was performed with an in-house allele-specific oligonucleotide (ASO) hybridization method using specific oligonucleotide probes for different HCV genotypes [genotype 1 (5'-CGC TCA ATG CCT GGA GAT-3'); HCV2a (5'-CAC TCT ATG CCC GGC CAT-3'), HCV2b (5'-CAC TCT ATA CCC GGC CAT-3'), HCV3 (5'-CGC TCA ATA CCC AGA AAT-3') and HCV4a (5'-CAC TCT ATG CCC GGC C-3)].

Genotypes 1 and 3 are predominant in patients from the Republic of Macedonia. Genotype 1 was found in 55.4% of genotyped patients (n = 714), while genotype 3 was found in 44.6% of genotyped patients (n = 632) [20]. There was statistically significant difference between the risk factors analyzed and the acquisition of HCV infection.

Hepatitis C virus is a highly prevalent infection in chronic dialysis patients (37.7%) and represents one of the major problems of hemodialysis units in our country [21]. Genotype 1 is predominant in this group of patients (90.7%). A combined infection (4.6%) of genotypes 2 and 3 was found in two patients (4.6%), and a combined infection of genotypes 1 and 3 was also found in another two patients (4.6%) [22].

We found predominance of HCV genotype 3 in HCV-positive IV drug users (93.35%). High prevalence of genotype 3 in an analyzed group of IV drug users is similar to the pattern of genotypes in IV drug users in both Europe and the USA [12]. The HCV genotype distribution in our patients over the years, shows a shift between the prevalence of genotypes 1 and 3. The increase of patients with genotype 3 is due to the increasing number of IV drug users analyzed at our center.

Evidence from several studies indicate that interferon signaling pathway genes *(IPGs)* and interferon stimulated genes *(ISGs)* are associated with

the host response to HCV infection. In order to investigate the possible association of six polymorphisms in the *OASL*-like interferon stimulated gene with a sustained virological response, we studied six single nucleotide polymorphisms in the *OASL* gene in two groups of patients with an HCV infection: patients who were non responders to the therapy, and those who had sustained a virological response to the therapy. Our preliminary results suggest that non ancestral alleles in four of the six studied polymorphisms in the *OASL* gene are associated with sustained virological response to pegylated interferon α-2a (Peg-IFN-α) plus ribavirin therapy [23].

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