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UP-TO-DATE EPIDEMIOLOGY OF CHRONIC HEPATITIS C VIRUS INFECTION AMONG BULGARIAN PATIENTS: A SINGLE CENTRE EXPERIENCE

Christo Pentchev, Mariya Petkova, Victor Alargkof, Yana Boyanova, Donika Todovichin, Aneta Ivanova, Assen Aleksiev, Krasimir Antonov

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Abstract

Epidemiological data about chronic infection with Hepatitis C virus (HCV) are limited in Bulgaria. The aim of this study was to determine the Viremic Rate and the distribution of HCV genotypes in one centre for diagnosis and treatment of chronic liver diseases. Geographic patterns of genotype distribution and associations of genotype with viral load, gender and age were investigated.

Serum samples from 1731 individuals with a positive HCV Antibody Test, collected between 2016 and 2019, were analysed. HCV RNA levels were determined by HCV quantitative RNA real time polymerase chain reaction. Individuals with detectable HCV RNA further underwent HCV genotyping analysis using the Versant HCV Genotype Assay (LiPA) 2.0.

The mean age of all patients was 52.9 ± 15.3 (17–87). Among those, 54.1% were men and 45.9% women. Most patients came from the south-western region of Bulgaria (21.7%). Viral load was proven in 1385 out of 1731 individuals (VR = 80%). Genotype 1 (G1) was the predominant genotype (89.8%), followed by G3 (8.5%), G2 (0.9%), G4 (0.6%) and G5 (0.1%). Among individuals with G1, infection with subtype 1b was seen in 77.4% and with subtype 1a in 22.6%. Viral load was not found to be dependent on age or gender. Viral load was higher in patients with G1 compared to G3 ($p = 0.001$). Finally, patients with G1b were significantly older than patients with G1a ($p < 0.001$) and G3 ($p < 0.001$).

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VR in the studied population (80%) appears to be higher than the VR reported for Central Europe (73.3%). In Bulgaria, the most prevalent genotype is G1 (89.8%). This is a higher rate in comparison to the observed G1 rate in Central Europe (70%). The second most prevalent genotype is G3 (8.5%), with fewer cases identified than in Central Europe (21%).

Key words: chronic hepatitis C, genotypes, viral load

Introduction. Hepatitis C virus (HCV) infection is one of the fundamental causes of liver-related morbidity and mortality, principally because of its progression. It is presumed that 71 million persons worldwide are infected with HCV [1]. The distribution of HCV genotypes displays geographic variations as well as exhibits changes over time.

Globally, the viremic rate (VR) is estimated to be 67%. In terms of genotype distribution, G1 is the most prevalent (49.1%), followed by G3 (17.9%), G4 (16.8%) and G2 (11%), with G5 and G6 making up less than 5%. Genotypes 4 and 5 are found predominantly in lower income countries [2].

Comparison of the age distribution of the infected population is difficult due to the wide variations in the year of gathered reports from different countries. Nevertheless, HCV infection generally appears to be rarer in persons younger than 20 years old and more prevalent in those aged more than 40 years old [3–5]. In countries where injection drug use (IDU) is the predominant source of new HCV infections,a higher prevalence is observed in men in younger age groups. On the other hand, in countries where infection is associated with an unsafe blood supply or nosocomial transmission, a higher prevalence is found in older age groups [6–8].

Countries from the group of North America, Canada and USA display a VR of 75.7%. The predominant genotypes are G1 (66.3%), G3 (15.7%), G2 (13.1%), and G4 (4.3%), with small percentages of G6 and no reported cases of G5. Differences can be seen among countries: G1 represents 60% of cases in Canada and 72.5% in USA, while G3 represents 22.3% in Canada and 8.9% in USA. Finally, evidence shows that in USA among the genotype 1 infection, 1a is more common than 1b [9].

HCV infection in Asia accounts for over 60% of the estimated cases worldwide. Genotype distribution displays high variability among the macro areas in Asia. The VR is 64.4%. The predominant genotype is G1 (46.6%), followed by G3 (22.4%), G2 (18.6%), G6 (7%) and small percentages of G4, G5 and mixed [2].

In Europe, the Global Burden of Diseases (GBD) subdivides the continent into three macro areas: Central, Eastern and Western. According to a literature review from 2000 to 2015 HCV VR is estimated to be 69.6% for Eastern Europe, 73.3% for Central Europe and 71% for Western Europe [10]. The predominant genotype is G1 (64.4%) followed by G3 (25.5%), G2 (5.5%), G4 (3.7%) and only small percentages of genotypes 5, 6, mixed and unclassified cases being reported. In general, genotype distribution does not show high variability between the macro

areas in Europe. Evidence suggests that the distribution of subtypes 1a and 1b depends on age and the route of transmission, with G1b being more common in older patients and G1a in people who inject drugs (PWIDs) [11, 12]. In contrast, G1b is more common than G1a in Europe.

Data from 2016 shows that for Central Europe, where Bulgaria belongs to, the predominant genotype is G1 (70.0%), followed by G3 (21%), G4 (4.9%) and G2(3.2%) with small percentages of mixed genotypes and G6, and no reported G5 cases.

According to the aforementioned literature review [10], no genotype distribution data were available for Bulgaria. In English literature, we could only identify a modelling study for the global prevalence and genotype distribution of HCV infection in 2015 that estimated the VR, anti-HCV prevalence and genotype distribution for Bulgaria. Other data regarding the epidemiology of HCV has only been published in Bulgarian literature so far [16, 17].

The aim of this study is to provide an update of the HCV infection data about the VR and genotype distribution in a group of patients from all major regions in Bulgaria. An additional aim was to determine the HCV genotype distribution patterns in the different geographic regions of Bulgaria and to investigate possible associations between genotype, VR, gender, and age. This data could ultimately aid guiding treatment options and prevention strategies in Bulgaria.

Materials and methods. We retrospectively analysed data from 1731 Hepatitis C Antibody Test positive subjects. All patients were referred to the Laboratory for Porphyrias and Molecular Diagnostics of Virus Induced Liver Diseases in "St. Ivan Rilski" Hospital, Sofia, for viral load testing in the period between January 2016 and December 2019. The mean age of all patients was 52.87 years (range 17–87, ±15.27). Nine hundred and thirty-seven were males (54.1%) and 794 were females (45.9%). The mean age of females was 57.49 years (range 17–86, ±15.071) and of males 49.23 years (range 18–87, ±14.66). The patients were residents of all five major geographic regions of Bulgaria. Analysis of HCV RNA levels was performed by HCV quantitative RNA real time PCR (polymerase chain reaction) using the Rotor-Gene Q System (Qiagen, Germany). The presence and quantification of HCV RNA was determined through: QIA symphony DSP Virus/Pathogen Midi Kit (Qiagen) according to manufacturer instructions; and the Artus HCV Q5-RGQ Kit (Qiagen). In addition, the Artus HCV Q5-RGQ Kit was used to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Orange of the Rotor-Gene Q. Results were reported in international units per millilitre (IU/ml). The test can quantify HCV RNA over the range of $65 - 1 \times 10^5$ HCV IU/ml.

The subjects with detectable HCV RNA further underwent HCV genotyping analysis using the Versant HCV Genotype Assay (LiPA) 2.0 (Siemens Healthcare Diagnostics). Genotypes and subgenotypes detectable by this test include 1, 1a, 2, 2a/c, 2b, 3, 3a, 3b, 3c, 4, 4a, 4b, 4c/d, 4e, 4f, 4h, 5a and 6a.

Statistical analysis was performed using SPSS for Windows, version 22. The results are expressed as means ± standard deviations or as percentages. The collected data was analysed using descriptive statistics, correlational analysis, the Mann–Whitney test and the Kolmogorov–Smirnov test. P values of 0.05 or less were considered statistically significant.

Results. Data concerning residency was available for 1138 out of 1731 patients. Most of the studied patients came from south-western 369 (33%), central south 315 (27.5%) and south-eastern 223 (19.4%) regions of Bulgaria. One hundred and thirty-three patients (11.6%) came from north-western areas, 52 (4.5%) from north-eastern and 47 (4.1%) from the central northern region of Bulgaria (Fig. 1).

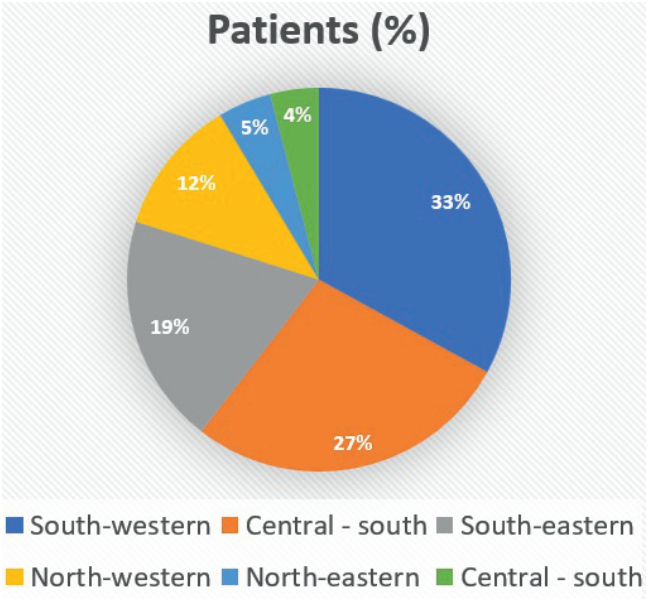


Fig. 1. Distribution by regions in Bulgaria

HCV RNA was detectable in 1385 of 1731 patients (VR = 80%). The mean age of all patients with active replication was 53.50 years ±15.43 (range 18–87). Among patients with active viral replication 53.65% were men with mean age 49.60 years ±14.80 (range 18–87) and 46.35% were women with mean age 58.40 years ±15.10 (range 18–86).

The mean viral load among patients with viral replication was $5.89 \log 10 \pm 6.19 \log 10$ IU/ml. The mean value of replication in men was $5.89 \log 10 \pm 6.12 \log 10$ IU/ml, and in women $5.88 \log 10 \pm 6.25 \log 10$ IU/ml. There was no statistically significant difference between mean viral load in male and female. The level of HCV RNA depends on the subgenotype ($p < 0.001$). Significant correlation was observed between mean viral load and age ($p = 0.005$) (Table 1).

Table 1

Correlation between age and mean viral load

Correlations

			HCV RNA	Age
Kendall's tau_b	HCV RNA	Correlation coefficient (r)	1.000	0.050**
		Sig. (2-tailed)		0.005
		N	1385	1385
	Age	Correlation coefficient (r)	0.050**	1.000
		Sig. (2-tailed)	0.005	
		N	1385	1385
Spearman's rho	HCV RNA	Correlation coefficient (r)	1.000	0.076**
		Sig. (2-tailed)		0.005
		N	1385	1385
	Age	Correlation coefficient (r)	0.076**	1.000
		Sig. (2-tailed)	0.005	
		N	1385	1385

The most prevalent genotype in our centre was found to be genotype 1, followed by genotype 3. The complete genotype distribution in our cohort is presented in Fig. 2. HCV genotype distribution by gender and viral load in the studied population is presented in Table 2.

Our results show that genotype 3 is more frequent in men than in women. In men, distribution of genotypes 1 and 3 was as follows: 85.9% for G1 and 12% for

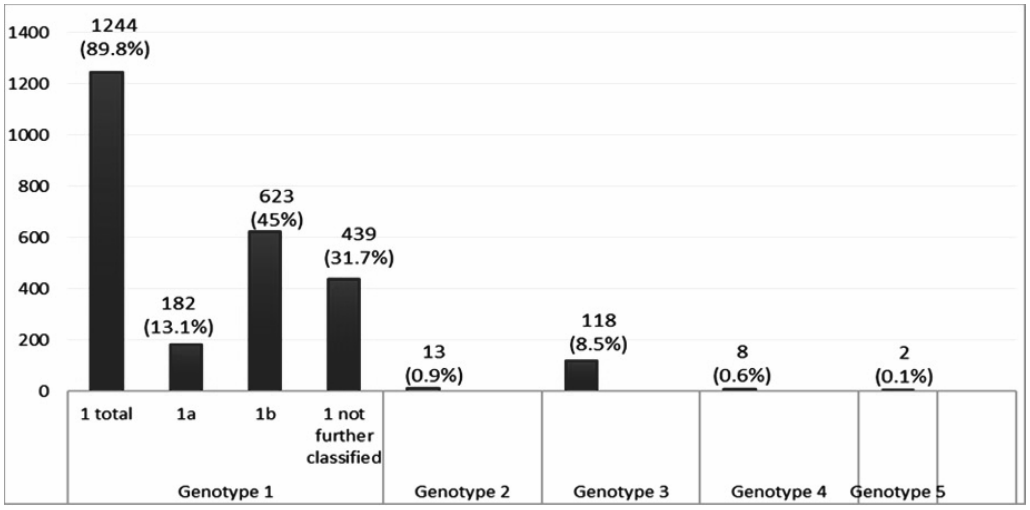


Fig. 2. Genotype distribution

Table 2

HCV genotype distribution by gender and viral load

HCV genotype	Gender		Viral load (log10 IU/ml)	
	Male *n* (%)	Female *n* (%)		
1	638 (85.8)	606 (94.4)	5.90 ± 6.2	
1a	118 (15.8)	64 (9.9)	5.90 ± 6.14	
1b	295 (39.7)	328 (51.1)	5.90 ± 6.35	
2	7 (1)	6 (0.9)	5.67 ± 5.68	
3	89 (12)	29 (4.5)	5.63 ± 5.85	
4	7 (1)	1 (0.2)	5.74 ± 5.99	
5	2 (0.2)	0 (0)	6.80 ± 6.77	
Total	743	642	5.88 ± 6.19	(2.14–7.43)

G3. In women, G1 was present in 94.4% and G3 in 4.5%. A statistically significant higher viral load was observed among patients with genotype 1 in comparison to patients with genotype 3 ($p < 0.001$). From the 1244 patients diagnosed with G1, 805 further underwent subgenotype analysis. One hundred and eighty-two patients were infected with subgenotype 1a (22.6%) and 623 with subgenotype 1b (77.4%). The mean viral load in patients with subgenotype 1a was 5.90 ± 6.14, mean age 39.74 ± 10.75 years, while in subgenotype 1b – 5.90 ± 6.35, mean age 58.45 ± 13.32. The G1a was more frequent in males, while in G1b there is no significant difference in distribution by gender.

Discussion. In neighbouring countries of Bulgaria specific features may be observed in respect to genotype distribution: In Romania G1 is almost the only genotype found (98%), a considerable percentage of G3 is described in the Republic of North Macedonia (44.6%) and a significant prevalence of G2 is described only in Albania (20%) [2].

A modelling study for the global prevalence and genotype distribution of HCV infection in 2015, utilizing a Delphi process, expert consensus and data from national surveillance systems or blood donor databases estimated the VR of Bulgaria to be 87%, anti-HCV prevalence as 1.5% and projected the following genotypes distribution: G1a (5.3%), G1b (72.3%), G3 (11.6%) and mixed/other (10.8%) [13–15].

The results of a study in Bulgarian that examined 107 asymptomatic individuals referred to the clinic of Gastroenterology in University Hospital "St. Marina", Varna, in 2011 with a positive anti-HCV antibody Test, showed the VR to be 83%. Another study in 2010 estimated the VR for Bulgaria to be 87%. After genotype analysis using PCR in 114 individuals with detectable HCV RNA it also found the most common genotype to be G1(82%), followed by G3(12%), G2 (2%) and reported 5% of mixed infection [16, 17].

Conclusion. According to our study, that includes infected with hepatitis C group of patients from all major regions of Bulgaria, genotype distribution is similar to that described in Europe. G1 is the most prevalent genotype. VR in the studied population (80%) appears to be higher than the VR reported for Central Europe (73.3%). In our study, the most prevalent genotype is G1 (89.8%). This is a higher rate in comparison to the observed G1 rate in Central Europe (70%). The second most prevalent genotype is G3 (8.5%), with fewer cases identified than in Central Europe (21%).

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Clinic of Gastroenterology, University Hospital "St. Ivan Rilski", Sofia, Bulgaria
Department of Internal Medicine, Faculty of Medicine, Medical University – Sofia
15 Akad. Ivan Evstratiev Geshov Blvd, 1431 Sofia, Bulgaria
e-mails: christopen4ev@gmail.com, mdpetkovamd@abv.bg, valargoss@yahoo.gr, boyanovay@gmail.com, donika.kysteva@gmail.com, anetaivanova99@abv.bg, assenalexiev@abv.bg, krasi_antonov@abv.bg