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Original Contribution

RESEARCH DATA ON THE PREVALENCE OF HEPATITIS C VIRUS ANTIBODIES AMONG INPATIENTS AT A HOSPITAL IN CENTRAL BULGARIA: 2021 – 2022

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ABSTRACT

Introduction and purpose: Hepatitis C Virus (HCV) infection is a leading cause of chronic liver diseases including cirrhosis and hepatocellular carcinoma. There is insufficient data on the prevalence of HCV among hospitalized patients. Targeted testing for anti-HCV is an effective strategy for identifying asymptomatic infected individuals. The purpose of the present study was to present the results of screening for anti-HCV among inpatients at a hospital in Central Bulgaria.

Methods: A total of 3275 serum samples from 1968 men and 1307 women were tested for presence of anti-HCV at the Laboratory of Virology within the Multidisciplinary Hospital for Active Treatment (MHAT) "Uni Hospital" (Panagyurishte, Bulgaria) in the period 2021-2022. Serological analysis based on the ELFA method was performed using VIDAS Anti-HCV automated test (BioMerieux, France).

Results: Thirty-two of the tested patients (1.0%; 95% CI 0.6%-1.3%) were HCV positive. The difference between the numbers of the hospitalized (n=29) and the outpatients (n=3) was notable. The sex distribution shows that 53.1% were men and 46.9% were women. Their median age was 67 years (IQR 56-75), as the involvement of the age group 60-79 years (n=16) was predominant. A total of eight patients were diagnosed with hepatic cirrhosis, whereas three more patients had hepatocellular carcinoma.

Conclusions: We found a low level of the prevalence of anti-HCV (1%) among the patients of MHAT "Uni Hospital", which is analogous to the level reported in the general population. We detected a higher incidence of HCV-positive serostatus among the older in comparison to younger age groups. Timely diagnosis and referrals to a gastroenterologist help to avoid progression of liver damage. All viremic patients (HCV RNA) require early access to modern antiviral therapy.

Key words: anti-HCV, prevalence, screening

INTRODUCTION

Hepatitis C is a socially significant infectious disease imposing health and economic burden on public health. Infection with *hepatitis C virus* (HCV) has a significant global impact. At a global scale chronic HCV infection is one of the leading causes of the development of hepatic cirrhosis and hepatocellular carcinoma (HCC). HCV-associated complications lead to permanent loss of working capacity and low survival rate. HCV accounts for 20% of cases of acute hepatitis and 70% of cases of chronic hepatitis, 40% of cases of end-stage cirrhosis, 60% of cases of HCC, and 30% of cases of liver transplant in developed countries (1). According to data by the World Health Organization (WHO) about 1.5 million new infections are established each year (2). Seroprevalence of HCV infection varies in different parts of world. The majority of countries from Western and Central Europe have relatively low rates of prevalence of HCV (3). Epidemiological data about chronic

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infection HCV are limited in Bulgaria (4). According to data by the European Centre for Disease Prevention and Control (ECDC) the national assessments of seroprevalence of anti-HCV in the general population vary from 0.1% to 5.9% (5). The highest rates are reported in Egypt (6.3%), Pakistan (3.8%) and Russia (3.3%), (6). An increasing incidence of registered cases has been recorded in developing countries and certain European regions (7). HCV can occur with different severity of liver damage and often asymptomatically until late clinical stages. Spontaneous viral clearance is rare (8). A high-grade viral persistence is characteristic. The mechanisms that determine the clearance or persistence of HCV have not yet been elucidated (9). Continuing chronic hepatic inflammation is associated with elevated risk for hepatic fibrosis, cirrhosis and HCC (10). The majority of people infected with HCV are undiagnosed and remain untreated which implies a serious risk for their health and hampers the fulfilment of the global WHO objective to eliminate hepatitis C (11). In the majority of cases infections are detected accidentally with prophylactic tests.

Laboratory diagnosis of HCV infection is a two-stage process. The sequence of examination includes an initial screening serological test of anti-HCV followed by a test of HCV RNA to confirm viremia (12). Antibodies to HCV are diagnostic markers for a prior exposure and do not have a protective effect. Specific molecular assays (Polymerase Chain Reaction, PCR) can determine whether a patient with positive result for antibodies to HCV has an ongoing (active) infection or a past resolved infection. Due to various reasons a significant share of the patients with anti-HCV fail to receive diagnostic PCR and are lost to follow-up (13). Screening for HCV is among the main strategic goals of WHO to reduce mortality and morbidity of HCV-associated chronic liver disease (14). Lost opportunities for active screening postpone timely detection of infected asymptomatic individuals, which prolongs the transmission of the virus in the community and obstructs the access to a timely and highly effective antiviral therapy (15). Treatment initiated in the earlier stages of infection is more effective and reduces mortality (16). The development of direct-acting antiviral agents (DAAs) has revolutionized treatment of hepatitis C with a very high rate of recovery and excellent safety profile (17).

The purpose of the present study is to present the results of screening for anti-HCV among inpatients at a hospital in Central Bulgaria.

MATERIALS AND METHODS

In the period 1 January 2021 – 31 December 2022, 3,275 patients within a wide age range (6 months – 88 years), including 1968 men and 1307 women, were tested in the Laboratory of Virology within the Multidisciplinary Hospital for Active Treatment (MHAT) “Uni Hospital”. All serum samples were tested via Enzyme Linked Fluorescent Assay (ELFA) – a technique combining EIA with fluorescent detection on VIDAS PC immunoassay analyser with a qualitative test VIDAS Anti-HCV (BioMerieux, France). The patients with positive results for anti-HCV were divided into five age groups by sex and age (≤19 years; 20–39 years; 40–59 years; 60–79 years; ≥80 years). In accordance with the requirements, a 3-5 mL volume of blood was collected for serological test in a prelabelled tube containing gel separator.

Statistical methods

The results are reported as numbers and proportions and median with interquartile range (IQR; 25th and 75th percentiles). The distribution was tested for normality with Kolmogorov–Smirnov test or Shapiro–Wilk tests. 95% CI with normal approximation to the proportion for binary variables was calculated. The averages of two groups were compared by Mann-Whitney U test. The relationship between categorical variables was assessed by Pearson chi-square test or Fisher’s Exact test. Statistical analysis was performed with SPSS v. 22.

RESULTS

Among the 3,275 studied patients, the relative proportion of men was higher (60.1%; 95% CI: 58.4% – 61.8%) than women (39.9%; 95% CI: 38.2% – 41.6%). Within the selected two-year period of follow-up (2021-2022) we found a low level of prevalence of anti-HCV antibodies among the patients of MHAT “Uni Hospital”: 32 patients, 1.0% (95% CI 0.6%-1.3%). The proportion of anti-HCV (+) males was 17/1968 (0.9%; 95% CI 0.5%-1.3%), whilst females were 15/1307 (1.2%; 95% CI 0.6%-1.7%). Despite the lower number of tested females, the proportion of anti-HCV (+) among them was higher, however without reaching a significant level (p=0.425).

The distribution by sex of the detected 32 patients with a positive HCV serostatus shows that 53.1%

are men and 46.9% are women (**Table 1**). Their median age is 67 years (IQR 56-75), and it is higher among females (68; IQR 58-76) compared to males (65; IQR 55-74) without any statistical significance (p=0.737). The age curves of both the sexes reflect the predominant involvement of patients in the age range of 60-79 years. In the youngest age group (≤19 years), anti-HCV seropositivity was demonstrated only in the 6-month-old male twins, who were born to an anti-HCV (+) mother.

Table 1. *Sex and age groups distribution of anti-HCV (+) patients*

	Males		Females		Total		
	n	%	n	%	n	%	95% CI
<20 years old	2	11.8	0	0.0	2	6.3	0.0%-14.6%
20-39 years old	0	0.0	1	6.7	1	3.1	0.0%-9.2%
40-59 years old	4	23.5	5	33.3	9	28.1	12.6%-43.7%
60-79 years old	10	58.8	6	40.0	16	50.0	32.7%-67.3%
80+ years old	1	5.9	3	20.0	4	12.5	1.0%-23.96%
Total	17	53.1	15	46.9	32	-	-

The anamnestic data of anti-HCV (+) patients were obtained through the hospital (laboratory) information system. Among the anti-HCV (+) patients, the number of hospitalized patients (n=29) was higher than the participating outpatients (n=3), and their median ages were significantly different due to the fact that the twins (6-months old) were outpatients, p=0.049 (**Table 2**). Within the specified period, the number of inpatients at the gastroenterology ward was the highest (n=12); a similar number of patients were tested preoperatively or prophylactically (before an invasive procedure) in the wards of vascular surgery (n=8) and cardiology (n=8), and one patient was treated in the oncology ward. Certain patients (5/12) were admitted in the gastroenterology ward presenting with complaints of heaviness in the right epigastrium, nausea, infirmity and fatigue as the reasons for their hospitalizations. Three of them had slightly to moderately elevated values of hepatic enzymes – AST in the range from 78 to 134 U/l (11-34 U/l), and ALT from 63 to 180 U/l (0-34 U/l), respectively.

Eight patients at the median age of 66.5 years were diagnosed with hepatic cirrhosis (**Table 2**), as one half of them had a mixed genesis (HCV + alcohol). They were evenly distributed between the two sexes (p=0.999).

Three men at the median age of 65 years had HCC. Three polymorbid men at the median age of 74 years, two of them with hepatic cirrhosis, deceased. Coinfections with hepatitis B virus (HBV) were confirmed in a 77- year-old men (HBsAg positive; HBV DNA 204 IU/ml) and a 59-year-old woman (HBsAg positive; HBV DNA negative) as they both were HCV RNA negative.

For the majority of patients with a positive HCV serostatus there was no epidemiological data on the probable mechanism of infection. Seven patients received antiviral therapy achieving sustained virological response (SVR). A total of seven patients reported Type 2 diabetes mellitus (T2DM) as comorbidity. The majority of patients were residents of Pazardzhik Province (n=29), 17 of them – residents of Panagyurishte Region (a city in Central Bulgaria), additional seven patients – residents of the Sofia City and Sofia Provinces, and nine patients – residents of other provinces.

Table 2. *Age and sex distribution by the categories of the patients*

		Age				Sex				
		Median	IQR		p	Males		Females		p
						n	%	n	%	
Sex	Males	65.0	55.0	74.0	0.737	17	100.0	0	0.0	-
	Females	68.0	58.0	76.0		0	0.0	15	100.0	
Status	Discharged	66.0	55.0	73.0	0.317	14	82.4	15	100.0	0.229
	Exitus	74.0	65.0	78.0		3	17.6	0	0.0	
Cirrhosis	No.	67.0	48.5	77.5	0.983	13	76.5	11	73.3	0.999
	Yes	66.5	59.0	72.0		4	23.5	4	26.7	
HCC	No.	68.0	55.0	76.0	0.808	14	82.4	15	100.0	0.229
	Yes	65.0	57.0	71.0		3	17.6	0	0.0	
Type of the patients	Hospitalized patients	68.0	58.0	76.0	0.049	15	88.2	14	93.3	0.999
	Outpatients	0.6	0.6	66.0		2	11.8	1	6.7	

DISCUSSION

The period of observation for this study was 2 years (2021-2022). The presented data reflects the results of serological screening of hospitalized patients and a small number of outpatients, who were referred to tests by general practitioners. According to the official data, Bulgaria is among the countries with a low rate of prevalence of HCV, which was also confirmed in our study (1%) including patients of MHAT "Uni Hospital" (Panagyurishte) in Central Bulgaria. A multi-center seroprevalence study, carried out in Bulgaria (covering the five largest cities – Sofia, Plovdiv, Varna, Pleven, and Stara Zagora) in 1999–2000 estimated a crude seroprevalence rate of 1.3% for anti-HCV and the results of other study (2010-2011) showed a low rate (0.7%) of HCV seropositivity among outpatients from the Plovdiv region (18). The overall positive percentage is similar to the levels of 0.7-1%, which was also reported in hospitalized patients in Turkey (19). A study among the patients of a hospital in Cairo (Egypt) showed a high percentage (19.8%) of the prevalence of antibodies to HCV (20). The majority of seroprevalence studies of the prevalence of the infection are based on the anti-HCV seropositivity. Anti-HCV antibodies are an accessible and major screening marker for a prior infection with the virus.

In Bulgaria, obligatory screening of donor blood was not performed until 1992. Observations show that the majority of HCV (+) individuals includes the cohort of people called "baby boomers", or those born in the period 1945-1965 (21). In Europe seroprevalence also increases with age. Data from Italy shows that 60% of cases of infection with the virus involve older people aged >65 years (22). The results of our study are similar. We detected a higher incidence of HCV-positive serostatus among the older in comparison to younger age groups. The involvement of the infected people in the group of 60-79 years for the two sexes is predominant. The median age of the detected patients with positive anti-HCV is 67 years, 53.1% of them and men and 46.9% are women. No difference associated with sex was seen in the number of patients with confirmed hepatic cirrhosis, which had a mixed (viral–ethylic) aetiology in 50% of cases. Both alcohol and HCV cause liver damage. Studies show that increased use of alcohol and HCV infection exercise a synergic hepatotoxic effect, and the simultaneous presence of the two factors increases the risk for progression of liver disease (23). Alcohol causes apoptosis, oxidative stress and immune dysfunction. In patients with chronic HCV infection, alcohol consumption increases the risk for developing cirrhosis and HCC (24). In our study, three male patients at an average age of 64.3 years had a confirmed HCC, as one of them showed anamnestic evidence for regular alcohol use. Out of the 32 patients with positive HCV antibodies, fatal outcomes occurred in three polymorbid men. In 22% of HCV positive patients, we found the presence of T2DM as comorbidity. HCV infection is associated with an elevated risk for T2DM irrespectively of the severity of an associated liver disease (25). Three times higher risk for DM was reported for individuals at the ages > 40 years with chronic HCV infection (26). Liver is the main site of glucose metabolism, and 30% of patients with cirrhosis have DM as a concomitant pathology (27). The predominant part of the patients with HCV-positive serostatus in "Uni Hospital" do not belong to conventional risk groups. None of the patients had a history of drug abuse. Two patients with end-stage chronic renal failure were treated with hemodialysis, and three patients at the age >70 years reported previous surgical interventions and blood transfusions. In the youngest age group, only two toddlers (twins) born to a seropositive mother had a positive result for anti-HCV antibodies. The extent of a vertical (maternal–neonatal) transmission is reported to be about 6% (28). Given the common ways of transmission of the infection, in two patients, a 59-year-old woman with cirrhosis and a 77-year-old man with steatosis, coinfections with hepatitis B were found.

In hepatitis C, a serological test for HCV antibodies is the first step in diagnostic algorithm followed by PCR assay for detection of viremia in the cases of reactive results. After the administration of a highly effective antiviral therapy, the successful control of the HCV infection requires the enhancement of screening to reduce the number of undiagnosed and untreated patients. The best possible variant in relation to diagnosis is a strategy of active screening (29). An important component in the comprehensive care of patients is their referral to gastroenterologist for a complete assessment of their condition, for providing antiviral treatment and post-therapeutic monitoring.

CONCLUSIONS

The overall anti-HCV seropositivity among the population of inpatients at "Uni Hospital" was 1%, which showed a low rate of prevalence of the infection in Central Bulgaria. The involvement of older age groups was predominant. Male sex, older age, presence of cofactors (alcohol, coinfection) can be associated with an elevated risk for complications. Hepatic cirrhosis was found in 25% of patients and HCC detected in 5%. Undiagnosed and untreated patients are exposed to higher risk for the disease progression. Among the global goals of WHO, the improvement of the system of screening and provision of a timely access to antiviral therapy is of high priority. Introduction of DDAs allowed hepatitis C to become the first curable chronic viral infection.

LIST OF ABBREVIATIONS

anti-HCV - antibody to hepatitis C virus
DAAs - direct-acting antiviral agents
ECDC - European Centre for Disease Prevention and Control
ELFA - Enzyme Linked Fluorescent Assay
HBsAg - Hepatitis B surface antigen
HBV - hepatitis B virus
HCC - hepatocellular carcinoma
HCV - hepatitis C virus
IQR - interquartile range
MHAT - Multidisciplinary Hospital for Active Treatment
PCR - Polymerase Chain Reaction
SVR - sustained virological response
WHO - World Health Organization

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