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ORIGINAL ARTICLE

PREVALENCE OF HEPATITIS B IN RANDOM BULGARIAN BLOOD SAMPLES – A SINGLE-SITE EXPERIENCE

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Abstract. *The worldwide distribution of the HBV infection has usually been estimated by the prevalence of hepatitis B surface antigen (HBsAg) among the general population. Total antibodies for hepatitis B core antigen (anti-HBc) are the most important diagnostic marker for proving prior exposure to HBV.* ***Aim:*** *The aim of this study was to determine the prevalence of Hepatitis B virus (HBV) infection among Bulgarian population. Reliable epidemiologic data is needed to estimate the prevalence of Hepatitis B in order to determine the actual burden of the infection and to plan prevention and control measures.* ***Materials and methods:*** *A total of 2140 serum samples were tested for major serological markers of HBV (HBsAg and anti-HBc) for a period of two years (2018-2019).* ***Results:*** *We established an intermediate prevalence of HBsAg (5.1%) and high prevalence of anti-HBc (27%) among HBsAg negative individuals.* ***Conclusion:*** *In conclusion, the prevalence of Hepatitis B is much higher among those born before the introduction of universal hepatitis B vaccination in Bulgaria (1992).*

Key words: *Hepatitis B, HBsAg, anti-HBc, prevalence, Bulgaria*

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INTRODUCTION

Globally, the prevalence of HBV infection in the general population is 3.5% and varies greatly in different parts of the world [1]. Approximately 75% of chronic carriers live in Asia and the Western Pacific. About 15 million people have chronic HBV infection in the European region of the World Health Organization (WHO). In 2017, 30 European Union/European Economic Area (EU/EEA) Member States reported a total of 26 907 cases of HBV infection [2]. Routine detection of infection with HBV has been based on the detection of hepatitis B surface antigen (HBsAg), which is the main screening serological marker. The presence of HBsAg indicates current HBV infection. The prevalence as well as endemicity of chronic HBV infection is defined by the presence of HBsAg and categorized as high (≥ 8%), intermediate (2-7%) and low (< 2%) [3]. Hepatitis B is highly endemic in Southeast Asia, China, sub-Saharan Africa and the Amazon Basin. Africa has the second largest number of chronic HBV carriers after Asia [4]. Hepatitis B is moderately endemic in part of Eastern and Southern Europe, the Middle East, Japan, and part of South America. The endemicity of HBV is low in most areas, such as North America, North and West Europe and Australia. The prevalence of HBsAg in the WHO European Region shows

distinct prevalence patterns ranging between 0.1% in Ireland and the Netherlands to > 7% in Eastern Turkey and is higher in south and central Europe (Turkey, Romania, Bulgaria and Greece) [5]. Knowledge of HBV's epidemiological pattern in each country is vital for establishing the risk for the population [6]. The predominant routes of viral transmission vary according to the endemicity of the HBV infection. Hepatitis B is mostly spread from mother to child at birth (perinatal transmission), or through horizontal transmission (exposure to infected blood) in highly endemic areas whilst in areas with low endemicity the infection is acquired primarily in adulthood through sexual transmission [7].

HBV is a hepatotropic virus. The infection can remain undetected for many years. Most adults develop acute hepatitis and fully recover. The likelihood of progression to chronic infection is inversely related to age at the time of infection. Around 90% of infants infected perinatally become chronic carriers [8]. In contrast, less than 5% of cases in immunocompetent adults become chronic [9]. Patients with chronic infection are often asymptomatic, but they are at high risk for serious health consequences, including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Hepatitis B is an important cause for chronic liver disease worldwide and results in substantial morbidity and mortality, predominantly through the consequences of chronic infection. There are between 600 000 and 1.2 million deaths yearly due to complications. HBV infection is the most prominent risk factor for HCC development, accounting for ~ 50% of cases [10]. More evidence suggest that HBV plays an important role in hepatocarcinogenesis.

The quantification of the burden of the infection with HBV requires knowledge on its prevalence in the general population. Current hepatitis B guidelines emphasize HBsAg and anti-HBc screening as a basic diagnostic approach for HBV infection. Chronic HBV infection is defined as persistence for more than 6 months of HBV surface antigen in the serum [11]. Hepatitis B core antibody (anti-HBc) is considered the most sensitive serological marker for history of HBV infection. Prevalence of HBsAg and anti-HBc is high in HBV endemic areas.

MATERIALS AND METHODS

The ultimate goal of the current study is to determine the prevalence of HBV among the general Bulgarian population via detection of major serologic screening markers of HBV infection: HBsAg and anti-HBc. For a period of two years (2018-2019) a total number of 2140 serum samples randomly picked from the general population were examined for the presence of HBsAg and anti-HBc. Subjects between 18 and 89 years of age were included. The larger part of them were males – 59.8% (95% CI: 57.7- 61.9%; n = 1279) and the females were 40.2% (95% CI: 38.2-42.4%; n = 861).

The examined persons were divided into 7 groups depending on their age as it's shown in Table 1. The average age of the participants was 53.3 ± 16.0 (54.1 ± 15.8 years for the male and 52.2 ± 16.3 years for the female, as the difference between them is significant, p = 0.009).

All serum samples were tested with LIAISON® (DiaSorin, Italy) assays, using chemiluminescence immunoassay (CLIA) technology for the qualitative determination of HBsAg and of anti-HBc in human serum or plasma samples. Testing was performed according to the instructions of the manufacturer. The analyst automatically classifies the results as positive or negative.

Table 1. Age groups distribution of the surveyed persons from the general population

Age groups (years)	Total number (n)	%	95% CI
< 30	158	7.4	6.3-8.6
30-39	313	14.6	13.2-16.2
40-49	405	18.9	17.3-20.7
50-59	438	20.5	18.8-22.2
60-69	481	22.5	20.7-24.3
70-79	248	11.6	10.3-13.0
80-89	97	4.5	13.7-5.5
Total	2140	100.0	

The examination of the serum samples was conducted while abiding the rules for good laboratory practice and keeping the personal information of the tested persons' confidentiality.

The examination of all serum samples was conducted in the Laboratory of Virology of MMA – Sofia.

Statistical analysis was performed by SPSS (SPSS Inc., IBM SPSS Statistics), version 22.0.

Qualitative variables were presented as number and percentage and quantitative as mean and standard deviation. Mean ages of two different groups were compared by t-test.

Pearson chi square test (Fisher's Exact test if applicable) was used to check the relationship between qualitative variables. Binary logistic regression was applied for searching significant factors of being in the group of people with HBV seroprofile: HBsAg(-) and anti-HBc(+). Values of $p < 0.05$ were considered as significant.

RESULTS

Prevalence of HBsAg

The proportion of HBsAg(+) individuals was 5.1% (95% CI: 4.2-6.2%; n = 110) and the proportion of seronegative individuals was 94.9% (95% CI: 93.8%-95.8%; n = 2030), respectively (Figure 1).

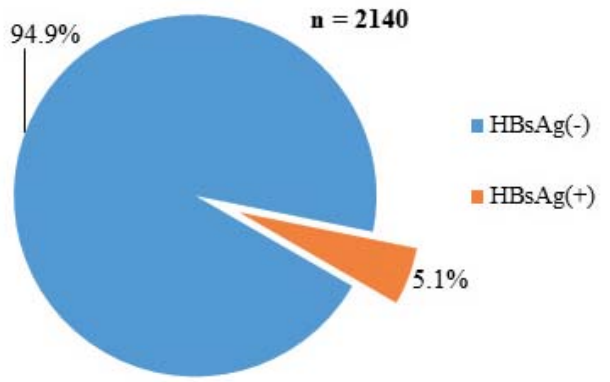


Fig. 1. Proportion of HBsAg (+) individuals in the general population

The distribution by gender showed that the proportion of HBsAg(+) among males was 5.1% (n = 65), while the relative share for females was similar – 5.2% (n = 45) (p > 0.05). The average age of the positive for HBsAg persons (n = 110) was 50.5 ± 15.1 years, while the average age of the negative for HBsAg persons was 53.5 ± 16.0 years. Splitting HBsAg(+) persons in seven groups by age showed a peak in the age range from 40 to 49 years which corresponds to data from available literature [14]. The results are shown in Figure 2.

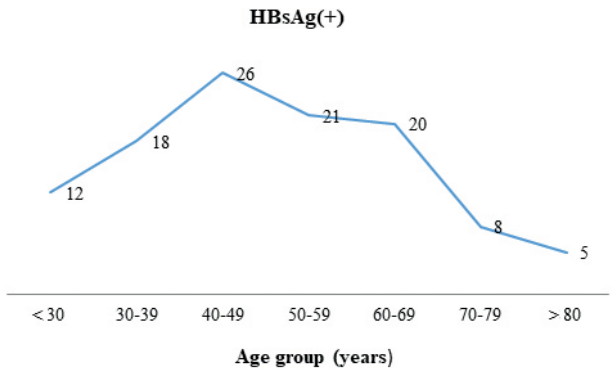


Fig. 2. Distribution of HBsAg by age groups among the 2140 tested individuals from the general population

The mandatory screening examination of pregnant women for carrying HBsAg as well as the key role of the vaccine-prophylaxis in limiting the spreading of HBV are discussed as possible reasons for the fewer positive amongst younger people (< 30 years).

Prevalence of anti-HBc in HBsAg negative general population

A total of 2140 serum samples of persons from the general population were examined in parallel for HBsAg and anti-HBc with the goal to assign the relative part of the people with HBV seroprofile: HBsAg(-)/anti-HBc(+). The prevalence of this seroprofile was 27.1% (n = 582) among the general population. The characteristics by gender revealed slightly higher frequency among males (28.2%; n = 361) compared to females (25.7%; n = 221), as the difference between them was not significant, p > 0.05 (Figure 3).

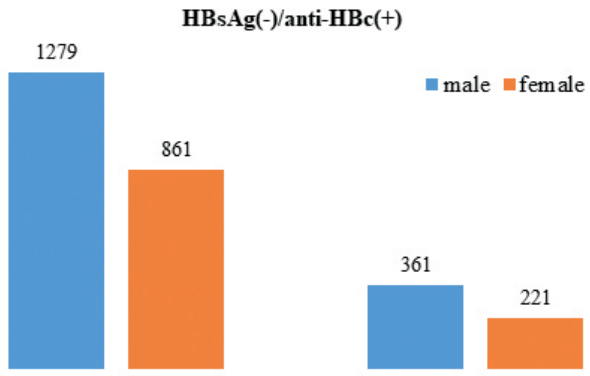


Fig. 3. Distribution of anti-HBc by gender in HBsAg negative general population

The number of the affected people in the age group < 30 years was the lowest one (n = 12) and it gradually increased and reached its peak in the age range from 60 to 69 years (n = 197), shown in Figure 4.

There was a similar spreading of people with the HBV seroprofile: anti-HBc(+)/HBsAg(-) in the age range from 50 to 59 years (n = 117) and 70 to 79 years (n = 110) in both genders as among the 80 years old there was a significant decrease (n = 28).

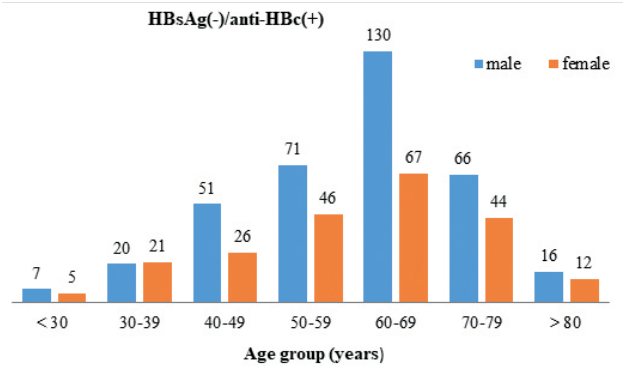


Fig. 4. Prevalence of HBV seroprofile: HBsAg(-)/anti-HBc(+) in different age groups

The prevalence of anti-HBc follows the trend of prevalence of HBsAg. The people with this HBV seropro-

file were at average age of 60.0 ± 13.3 while the average age of the rest was 50.9 ± 16.3 as the difference between them was significant ($p < 0.001$).

Age was found to be a significant factor for the presence of HBV seroprofile: HBsAg(-)/anti-HBc(+) as for every one year increase in age, the chance of a positive result increased by 3.8%, hazard ratio (HR) = 1.038 (95% CI 1.032-1.045), $p < 0.001$.

DISCUSSION

The current study found that 5.1% of the studied general population was positive for HBsAg. According to the criteria of WHO (2-7%) this result confirms the intermediary prevalence of HBsAg in Bulgaria and is similar to the reported data by other researches.

The data for Bulgaria confirms that the prevalence of HBsAg is between 3% and 7% which puts the country in the intermediate endemic zone [12].

Research conducted in 2011 registered seroprevalence of 3.8% with variations in the different regions in the country from 1.9% to 5.3% [13].

By analysing the gender and age-specific distribution of HBsAg in the general population, we found no significant difference in the relative parts of HBsAg(+) people between the two genders ($p > 0.05$). Moreover, the age as a factor is with a borderline significance ($p = 0.055$). The increase in the proportion of HBsAg(+) people with aging is a result of risk exposure accumulation (sexual attitude, narrow rustic contact or the so called horizontal way of infecting in families with HBsAg(+) person, lack of vaccine). This is also the explanation why people in sexually active age are predominantly being affected. Household members and sex partners of HBsAg-positive persons should be tested for HBsAg.

Apart from the HBsAg, anti-HBc is a screening marker and an indicator for the prevalence of HBV in the general population. The most reliable serological markers of HBV infection are called its epidemiological markers. Our results show high spreading (27.1%) of HBV seroprofiles: HBsAg(-)/anti-HBc(+) among the people form the general Bulgarian population and the results are comparable with the results from two other Balkan countries – Romania (27%) and Turkey (21.1%) [15]. According to other authors the proportion of anti-HBc(+) people in the general population in our country is between 17.8% and 38.6% and varies within regions [16, 17].

Our results confirm the tendency that the proportion of antiHBc(+) people among general population increase with age. About one-third of the world's population has serological evidence of past or present HBV infection according to the European Association for the Study of the Liver (EASL) [18]. Anti-HBc is the most constant marker of past infection with the virus and may be the only serological marker of HBV infection. The interpretation of isolated antiHBc(+) result is difficult because it refers to the presence of anti-HBc in the absence of HBsAg and anti-HBs. It is observed relatively frequently among high-risk groups (immunocompromised individuals, intravenous drug users, hemodialysis patients and patients with HCV or HIV coinfection) [19].

Anti-HBc may be detected even in the absence of HBsAg and this serological pattern is known as „isolated anti-HBc". Isolated anti-HBc indicates previous infection or resolved HBV infection, chronic infection with low levels of replication and in some rare cases can be a false positive result [20]. Their presence among HBsAg negative patients can indicate also occult (hidden) hepatitis B infection (OBI). Thus, hepatitis B screening without inclusion of anti-HBc can potentially miss a significant number of individuals with OBI and/or a history of past infection in endemic populations. OBI is an unusual form of HBV infection that causes a dissimilar pattern of serological markers. Occult hepatitis B infection (OBI) is defined as the existence of low-level HBV DNA in the serum, cells of the lymphatic (immune) system, and/or hepatic tissue in patients with serological markers of previous infection (anti-HBc and/or anti-HBs positive) and the absence of serum HBsAg [21]. According to the literature the spreading of OBI is estimated between 4% and 25% in the anti-HBc(+) people [22]. OBI can have continuous effects on the infected persons [23]. Patients with OBI who are subjected to immunosuppression are at higher risk of infection reactivation [24]. OBI leads to an increased risk of chronic disease and further transmission of the virus mainly by hemotransfusion or organ transplantation [25]. Antibodies to hepatitis B core antigen may be used in prevaccination testing to determine previous exposure to HBV infection. It's important all negative patients to get a recommendation for hepatitis B vaccine. Vaccination is the most effective strategy to diminish the spread of HBV in the community. Universal childhood immunization with three doses of hepatitis B vaccine in the first year of life is the most effective measure for the prevention of hepatitis B. Among children less than five years of age, the prevalence of chronic HBV infection is less than 1%. The lower prevalence in the paediatric population reflects the effectiveness of the global vaccination program against viral hepatitis B.

CONCLUSION

According to WHO more than two billion people are HBV infected worldwide which makes hepatitis

B one of the most common infection in the world. The prevalence of HBV varies from country to country and among the general population in Bulgaria is intermediate for HBsAg and high for anti-HBc, both increasing with age. Updated epidemiologic data provided to the authorities can improve surveillance and hepatitis B control programs. The major measure for HBV infection prevention is the vaccination. HBsAg and anti-HBc negative people should be immunized with hepatitis B vaccine. Untreated infections could lead to cirrhosis and HCC.

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