PREVALENCE OF ANTI-HBC IN HBSAG-NEGATIVE POPULATION: SCREENING OF PATIENTS WITH UNSPECIFIED ACUTE HEPATITIS AND REVIEW OF THE LITERATURE

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ABSTRACT

Background: Among the various serological markers employed in the hepatitis B virus (HBV) differential laboratory diagnosis, serum HBsAg is considered the most reliable. In order to characterise the HBV infection, another important diagnostic marker is employed – the HBc antibody (anti-HBc). There are three categories of anti-HBc-positive individuals: patients with HBV immunity, patients with chronic HBV and individuals with the so-called isolated anti-HBc pattern. The current study aimed to evaluate the presence of anti-HBc in patients negative for HBsAg whose clinical diagnosis was acute viral hepatitis.

Material and methods: A total of 88 specimens were examined, of which 75 sera were from prospective patients diagnosed with acute viral hepatitis, and 13 sera from breast milk donors. Antibodies against the hepatitis B core antigen were detected by enzyme-linked immunosorbent assay (ELISA).

Results: Twenty-eight (32%) of all tested samples were positive for anti-HBc. Nineteen samples belonged to male and 9 to female patients. One positive sample was from a breast milk donor. Two age groups, namely 46-55 years and 56-65 years, demonstrated the highest rate of anti-HBc positivity. Among the other age groups positivity rates varied from 15% to 36%. The results demonstrated a linear trend of increasing anti-HBc prevalence with increasing age.

Conclusions: Considering the highest rate of anti-HBc positivity being demonstrated in the age range 46-65 years, it could be assumed that a sufficient number of risk factors accumulate over time resulting in greater population susceptibility to HBV infection.

KEYWORDS:

HBV, anti-HBc, acute hepatitis

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INTRODUCTION

Hepatitis B virus (HBV) is a causative agent of acute or chronic viral infection that represents a major public health problem with significant morbidity and mortality. More than 500 000 newly infected cases are reported each year. According to the World Health Organisation (WHO), about 248 million people are chronically infected, and approximately 686 million deaths per year are due to secondary complications of HBV infection such as hepatocellular carcinoma and cirrhosis (1).

The worldwide distribution of the virus has traditionally been determined by the seroprevalence of the surface antigen (HBsAg) in a given population. Highly endemic countries demonstrate HBsAg prevalence greater than 8%. In countries with intermediate endemicity, the HBsAg prevalence is subdivided into lower-intermediate (2-4.99%) and higher-intermediate (5-7.99%) and, finally, in low endemic areas, less than 2% of the population is affected (2). In hyper-endemic regions, the vast majority of cases is comprised of individuals that were infected perinatally or later in early childhood through horizontal transmission. Perinatal transmission is also possible in areas with intermediate endemicity, but the primary mode of transmission remains horizontal. In hypo-endemic areas, infections tend to be incidental and are most commonly the result of unprotected intercourse, injecting drug use or other unsafe exposure to blood products (3, 4). In a large multicentre study on the presence of major HBV markers among the general population in Bulgaria during the period 1999-2000, HBsAg prevalence was found in 3.87% of

the total population, i.e. Bulgaria falls into the group of intermediate endemicity with respect to HBV (5)**.** In 2017, a total of 3132 cases of viral hepatitis were reported in Bulgaria, and the rate of viral hepatitis B was 7.95%, meaning that 249 cases were reported in 26 districts of the country – Stara Zagora with 12.14%$_{000}$ morbidity, followed by the districts of Pernik (9.70%$_{000}$), Sliven (6.85%$_{000}$), Montana (6.68%$_{000}$) and Gabrovo (6.23%$_{000}$). In 22 (8.83%) of the reported cases, the affected were aged 19 years or less and 12 (54.55%) of the patients had been previously immunised against hepatitis B (6). In accordance with Ordinance No. 21 on the Procedure for Registration, Reporting and Control of Infectious Diseases, the acute form of viral hepatitis B is subject to mandatory registration and reporting (7)**.**

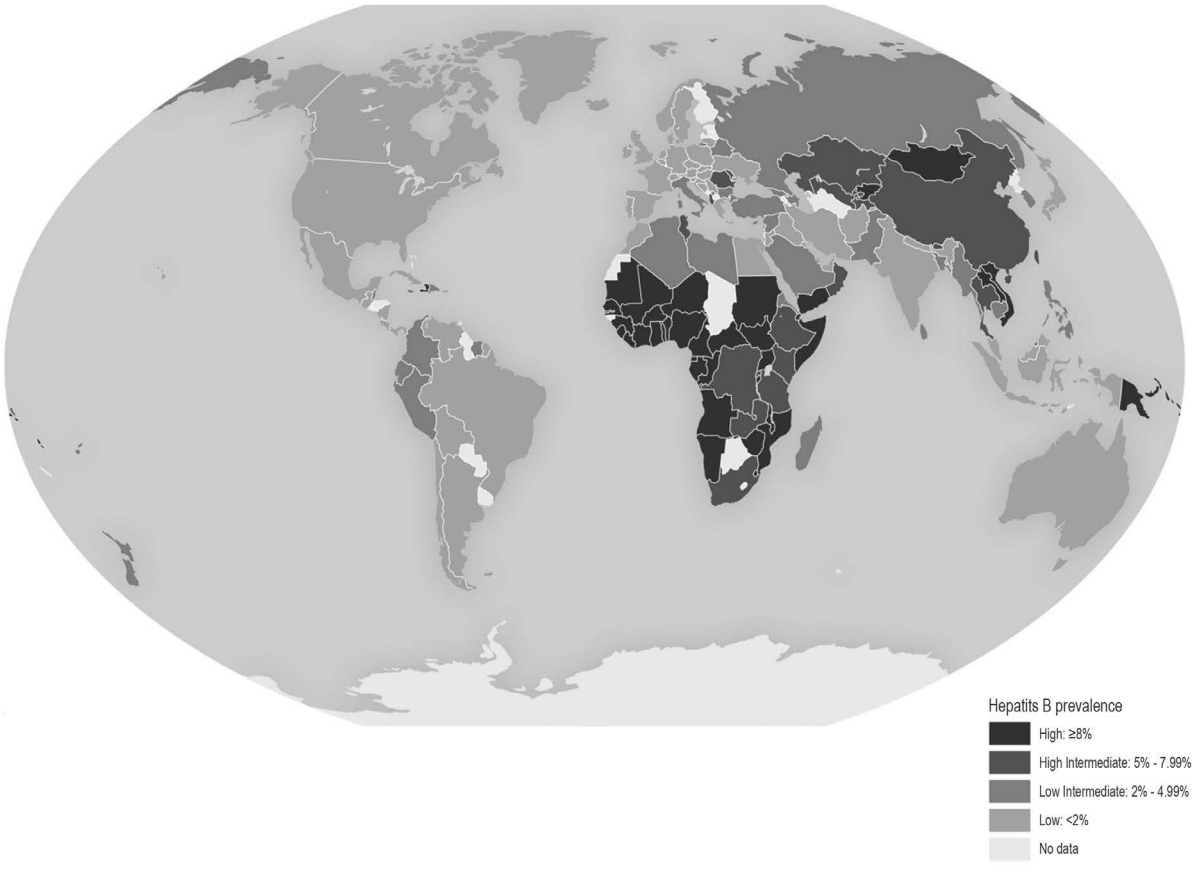

Figure 1. Prevalence of hepatitis B virus infection estimated from data on HBsAg antigenemia. (Source: Centres for Disease Control and Prevention. Infectious Diseases Related to Travel: Hepatitis B, https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/hepatitis-b)

Hepatitis B virus can be found in almost all bodily fluids. However blood, semen and vaginal secretions have the greatest infectious potential, and thus, transmission occurs through percutaneous or mucosal exposure to infective fluids (8). The virus is not found in urine, sweat and stool. Serological tests for the detection of HBV antigens and antibodies are the mainstay of diagnostic screening and utilise blood with subsequent serum or plasma isolation. Both HBV antigens and antibodies are stable for days at room temperature, for months at -4°C and years if stored at -20°C to -40°C.

Following infection, the first marker to appear in the blood circulation is the HBV surface antigen (HBsAg**)**, which becomes detectable 2-4 weeks prior to biochemical evidence of liver damage or the onset of jaundice (9**)**. This is the main viral protein that induces protective immunity. The marker becomes negative upon infection elimination after a period of 1-2 months and its persistence beyond 6 months is an evidence for chronic infection (9, 10). The HBsAg loss is marked by the development of protective antibodies – anti-HBs. The presence of anti-HBs only is evidence for vaccination. An anti-HBs serologic test result

demonstrating more than 10 mIU/mL is indicative of protective immunity (11). Some patients may be positive for both HBsAg and anti-HBs implying ineffective virus neutralisation and a chronic carrier state. Another specific HBV viral protein that could be found in serum is the HBV e-antigen (HBeAg). Since HBeAg is associated with the acute phase of HBV infection, i.e. with high levels of viral DNA, it is an indicator of a high degree of replication and infectivity. Seroconversion to anti-HBe may occur after years in patients with chronic HBV infection. The disappearance of HBeAg, even without seroconversion, is a sign of a significant decrease in the viral titre. Some HBV variants carrying certain mutations do not produce HBeAg or produce very low levels (pre-core and core mutants), but continue to have high HBsAg levels and are associated with poor clinical prognosis, i.e. prolonged and severe disease with a substantial risk of cirrhosis (10). Another important diagnostic marker is the HBV core antigen **(**HBcAg**)**, which is essentially a capsid protein. It is not readily detectable in serum as the antigen is incorporated into the virion, but it is highly immunogenic and its antibody (anti-HBc) is detectable in blood with the onset of clinical symptoms. The IgM class antibodies against HBcAg (anti-HBc IgM)**,** are the first to appear approximately 6 weeks after HBV exposure. The high titre of anti-HBc IgM may be the only marker detectable in the "window" period of acute HBV infection prior to seroconversion of HBsAg to anti-HBs (12).

In recent years, the emphasis has been placed on the extent to which HBsAg tests alone can be relied upon for acute or chronic HBV infection diagnosis. Serum HBsAg detection is considered a reliable marker of HBV infection, but it is insufficient to differentiate between an inactive carrier state, acute or chronic HBV infection (13). False-negative results during the window period, false-positive results, occult infections, and the presence of HBsAg mutants that cannot be demonstrated by standard assays have to be taken into consideration (14). A particular case is fulminant hepatitis, where HBsAg disappears too early due to the fulminant course of the disease, which also delays seroconversion to anti-HBs (15). Typical example is a study of 27 cases of fulminant hepatitis, conducted by Shimizu et al., of which 11 patients demonstrated both HBsAg and anti-HBc IgM but were initially diagnosed by testing for anti-HBc IgM. For the remaining 9 patients from the same study, anti-HBc IgM titres were demonstrated without detecting HBsAg (16).

Detection of HBsAg, anti-HCV, anti-HAV IgM and before the year 2020 occasionally detection of anti-HEV IgM, was requested for differential diagnosis of patients with acute viral hepatitis. In the National Reference Laboratory (NRL) "Hepatitis viruses" in case of HBsAg-positive results, samples were evaluated for the presence of anti-HBc IgM and/or HBeAg for confirmation of acute HBV infection. The present study further analysed the prevalence of anti-HBc total in 75 patients and 13 control subjects (breast milk donors) with the main aim to evaluate the presence of anti-HBc in patients negative for HBsAg whose clinical diagnosis was acute viral hepatitis.

MATERIAL AND METHODS

Seventy-five sera of prospective patients diagnosed with acute viral hepatitis as well as 13 sera of breast milk donors (as negative controls) were investigated in the present study. Samples collected from January to December 2016 were selected from sera bank of the NRL "Hepatitis viruses". All samples were tested previously in the NRL "Hepatitis viruses" and met the criteria to be negative for HBsAg, anti-HBc IgM, anti-HAV and anti-HCV. For *in vitro* detection of anti-HBc total in human sera a qualitative enzyme-linked immunosorbent assay (ELISA) ANTICORASE B-96 (TMB) (General Biologicals Corporation) was employed according to the manufacturer's instructions. The diagnostic specificity and sensitivity of the test are 99.8% and 100%, respectively. Specimens with absorbance values greater than 1.1 multiplied by the cutoff value are considered negative for anti-HBc. Specimens with absorbance values less than 0.9 multiplied by the cutoff value are considered positive for anti-HBc. All positive specimens along with specimens with absorbance value falling within the retest range, which is the cutoff value ± 10%, were retested and interpreted as above. The tested samples were divided into 6 groups depending on the age of the patient: patients between 15 and 25 years; 26 to 35 years; 36 to 45 years; 45 to 55 years; 56 to 65 years and patients between 66 and 75 years old.

RESULTS

For the period January-December 2016 in the NRL "Hepatitis viruses" 1802 samples were received for differentiation of viral hepatitis or for screening of hepatitis markers. From these samples, 1356 (75%) were tested for the presence of HBsAg and 190 (14%) were positive; 1019 (57%) were tested for anti-HCV and 127 (12%) were positive; 306 (17%) were tested for anti-HAV IgM with 84 (27%) positive results; and 501 (28%) were tested for the presence of anti-HEV IgM and 162 (32%) were positive.

During a training practice conducted in the NRL "Hepatitis Viruses" at the National Centre of Infectious and Parasitic Diseases, within the framework of the "Student Practices – Phase 1" project implemented by the Ministry of Education and Science in partnership with higher education institutions and scientific organisations in Bulgaria, blood serum samples were screened for the presence of anti-HBc. The studied patients were from Sofia, Pernik, Gabrovo, Haskovo and Shumen. The breast milk donors were from Sofia only. All tested samples met the selected criteria to be HBsAg-, anti-HBc IgM-, anti-HAV- and anti-HCV-negative and to be with sufficient quality and quantity for testing. The mean age was 40 years ± 15, the youngest individual being 15 and the eldest – 69 years of age; male to female ratio was 46:42. For 8 patients the age was not recorded in the medical profile. With regard to anti-HBc presence in sera, 28 (32%) of all tested samples were positive. From the positive samples 19 were male and 9 female patients. One positive sample belonged to a breast milk donor. Eighteen of all positive samples were from patients from Sofia, 7 from Pernik, and 1 from Gabrovo, Haskovo and Shumen, respectively. We evaluated the prevalence of anti-HBc total among the different age groups. Three age groups demonstrated the highest rate of anti-HBc positivity – 46% for the age group 46-55 years, 50% for 66-75 years and 55% for 56-65 years (Fig. 2).

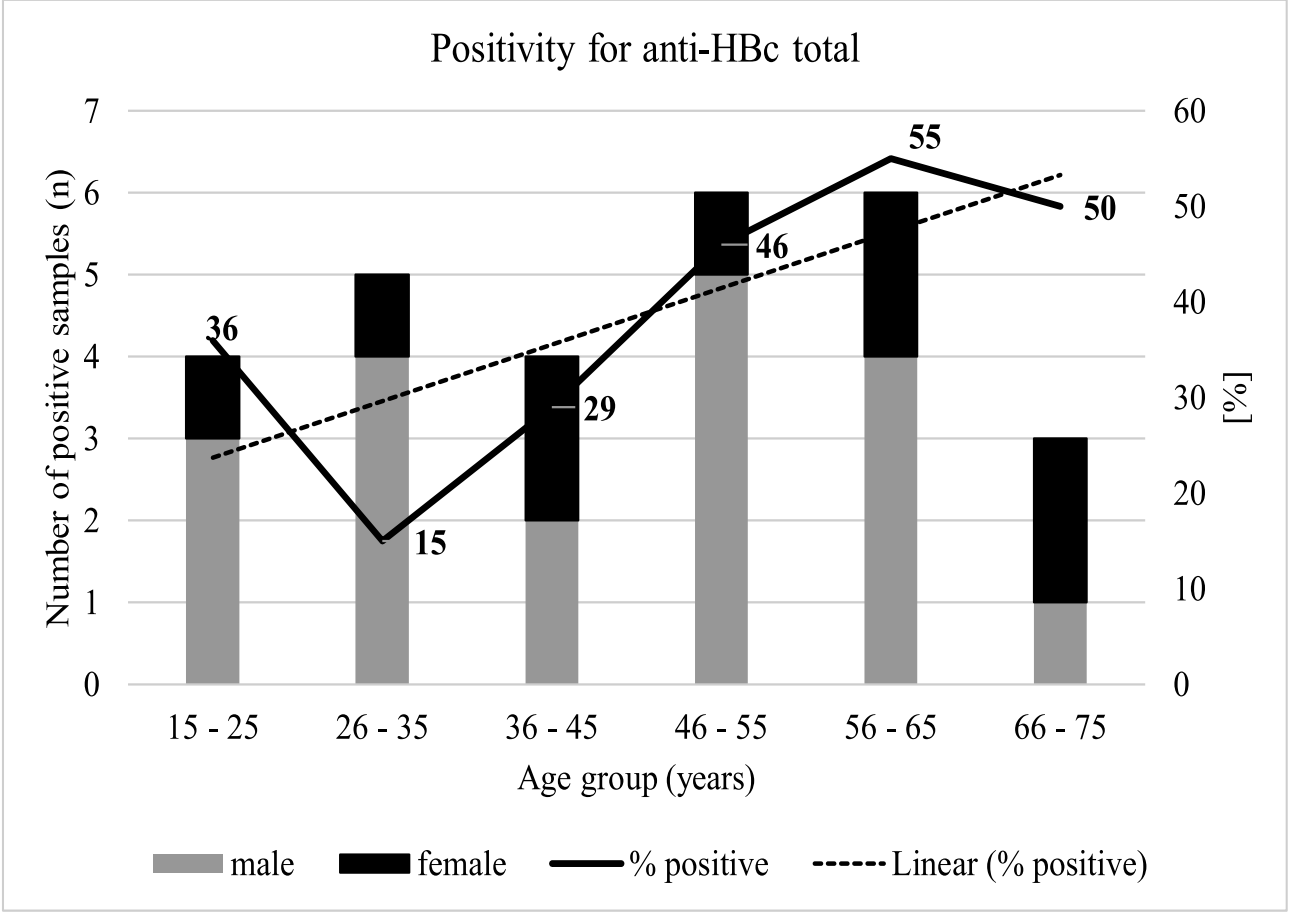

Figure 2. Age distribution of anti-HBc-positive individuals.
Legend: Percentage refers to a proportion of positive individuals for each age group.

Among the other age groups, positivity varied from 15% to 36%. In the present study, the peak of anti-HBc positivity is in the active age over 45 years, with males being predominantly anti-HBc-positive except for two age groups, namely 36-45 years and 66-75 years.

DISCUSSION

The current study revealed that 32% of the studied population were positive for the presence of anti-HBc total with prevalence in male individuals. The results demonstrated a linear trend of increasing anti-HBc prevalence with increasing age. Similar results were reported in the multicentre study conducted in Bulgaria in the period 1999-2000 (5). The apparent steady increase in seropositivity with age confirms the fact that a sufficient number of risk factors and occasions for exposure accumulate over time rendering the population susceptible to HBV infection. Given that anti-HBc antibodies remain in the serum for a significantly long period of time and are indicative of HBV exposure, they are suitable as a screening test for blood donation or for distinguishing whether a patient has acquired immunity through exposure or vaccination. These antibodies may be the only serological marker for HBV infection and potentially infectious blood.

Individuals who show no sign of liver disease but are anti-HBc-positive can be divided into three categories: 1) those with HBV immunity, i.e. individuals who are anti-HBc- and anti-HBs-positive and HBsAg-negative; 2) individuals with chronic HBV that are HBsAg-positive, and 3) individuals with the so-called isolated anti-HBc pattern (IAHBc) – they are positive for anti-HBc and negative for both anti-HBs and HBsAg (17). The IAHBc pattern is not unusual given that it is reported in 10-20% of patients with positive serology for HBV without the presence of other serological markers for HBV infection (18). This serological profile is often observed in intravenous drug users, hepatitis co-infections and pregnant women (18, 19). In HIV-positive patients, IAHBc occurs in 7% to 40% of the cases (20). In the evaluation of the IAHBc serological profile, several interpretations may be considered, of which the most significant are the window period in the acute infection, a false-positive result, past or occult HBV infection (21, 22). During the acute phase of HBV infection, it is impossible to detect HBsAg and HBeAg and their corresponding antibodies anti-HBs and anti-HBe in serum by conventional tests due to the formation of immune complexes which underlies the serological profile of isolated anti-HBc (19, 21). The IAHBc pattern could be observed years after recovery of the patient owing to a decline in the anti-HBs titre (21). This profile is also observable in chronic infections with waning anti-HBs titre or in the presence of HBsAg escape mutants (19, 23). Patients who are carriers of anti-HBc alone should be retested to rule out false-positive results (21). These cases are more commonly encountered in low prevalence areas and result from non-specific cross-reactivity reactions, circumstances related to the applied detection method and technical preparation. The primary mechanism that explains the IAHBc serostatus in HCV co-infected individuals is inhibition of HBV replication by the direct effect that the HCV core protein exerts on HBV (19). Another study suggests an alternative mechanism in which a stronger immune response to HBV leads to the formation of anti-HBs with partial clearance of HBV and partial HCV suppression. The subsequent loss of anti-HBs due to possible cross-reactivity and other mechanisms is a prerequisite for reactivation of HCV causing active HCV infection with IAHBc serostatus (24). Carriers of isolated anti-HBc ought to be considered potentially infectious, as instances of HBV transmission from blood or grafts from anti-HBc only-positive donors have been reported (22, 23, 25, 26).

In conclusion, the prevalence of anti-HBc in the studied population increases with age. It could be assumed that a sufficient number of risk factors accumulate over time resulting in greater population susceptibility to HBV infection. We should note the main limitation of the study – the small number of samples tested; however, the study was carried out in the framework of a project with the main purpose to familiarise medical students, under the supervision of a mentor, with the laboratory differential diagnosis of hepatitis viruses and the activity of NRL "Hepatitis viruses". The primary focus was the acquisition of practical and analytical skills while working in a laboratory setting and with scientific literature to design and conduct a study leading to publication of a scientific article.

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