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Original article

HEPATITIS B VACCINATION STATUS AMONG A TARGET GROUP BORN IN 1992-2000: A STUDY FROM BULGARIA

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ABSTRACT:

Hepatitis B virus (HBV) infection is a significant cause of morbidity and mortality and a leading cause of chronic liver disease. Vaccination has a key role in hepatitis B prevention. Compulsory immunization for all healthy newborns was introduced in Bulgaria in 1992 as part of the WHO global strategy.

Purpose: The aim of this study was to determine the extent of post-vaccination seroprotection among persons born in 1992 – 2000 who received recombinant hepatitis B vaccine.

Material/methods: A total of 923 serum samples of a target group of vaccinated individuals (412 males and 511 females) at a mean age of 23.0±2.7 years were tested over a two-year period (2018-2019). The quantitative analysis of hepatitis B surface antibodies (anti-HBs) levels was performed by CLIA using a LIAISON® anti-HBs II quantitative diagnostic kit (Dia Sorin, Italy).

Results: All 923 individuals included in the study were hepatitis B surface antigen (HBsAg) negative. Protective anti-HBs titers ranging from 11 to >1000 mIU/mL were found in 45.3% of them. The sex distribution of the tested subjects varied significantly between years (p=0.023). The difference in protective anti-HBs levels between the two sexes was non-significant (p>0.05). There was a weak negative correlation between year of birth and anti-HBs titer (Pearson's r=0.351, p<0.001).

Conclusions: The results of this large study conducted among subjects immunized against hepatitis B in childhood showed varying levels of post-vaccination seroprotection. On average, 23 years after universal immunization, 54.7% of the study cohort had no protective levels of anti-HBs (negative or equivocal result).

Keywords: hepatitis B virus, hepatitis B antibodies, hepatitis B surface antigen, vaccines, vaccine-preventable diseases,

INTRODUCTION

Hepatitis B is an important cause of global morbidity and mortality. Over 2 billion people are infected with hepatitis B virus (HBV) worldwide, with 820,000 deaths in 2019 alone. Chronic infection can remain asymptomatic for a long time but is associated with an increased risk of hepatic cirrhosis and hepatocellular carcinoma (HCC). Approximately 50-80% of HCC cases are of HBV etiology [1]. Vaccine prophylaxis plays a key role in the prevention of the disease. Vaccination remains the most effective and cost-effective measure for successful control despite the availability of antiviral therapy [2, 3]. In countries with a high prevalence of HBV, the leading route of infection is maternal-neonatal transmission, and infected children become chronic carriers in 90% of cases. Individuals who are positive for the Hepatitis B surface antigen (HBsAg) are a reservoir and source of infection. Effective vaccines have been available since the 1980s. Their role in limiting the spread of infection and reducing its clinical burden has been demonstrated. The WHO approved the integration of the HBV vaccine into national immunization programs in 1991. The three-dose vaccine coverage reached 85% globally in 2019, compared with about 30% in 2000 [4]. As a direct reflection of the impact of routine immunization, there has been a significant decline in HBsAg prevalence in childhood. Observations have confirmed the epidemiological, immunological and cost-effectiveness of the hepatitis B vaccine [5]. The recombinant HBV vaccine became mandatory in the National Immunization Calendar of Bulgaria in 1992 [6]. Prior to that, selective immunization of newborns from HBsAg positive mothers had been conducted in our country in the period 1988-1991. After this period, a decreasing trend was reported, with an 82% reduction in the incidence of hepatitis B among infants [7].

Protective immunity after vaccination is the result of an induced humoral and cellular immune response. Specific anti-HBs antibodies provide humoral protection. Long-term protection is associated with immune memory, determined by memory T cells, and persists for a long time [8]. The post-vaccination immune response is assessed via quantitative analysis of anti-HBs antibody levels (titer). Typically, anti-HBs levels of >10 mIU/mL are considered

protective and are defined as a positive response to the vaccine. The goal of mass immunization is to achieve high vaccine coverage. Vaccine coverage is measured after completion of a full course of three doses of vaccine. Many long-term observations have assessed the duration of seroprotection and the need the administration of additional doses. Studies suggest that post-vaccine protection is long-lasting [9]. An anamnestic immune response after a booster dose of vaccine confirms the existence of immunological memory in immunized individuals [10].

PURPOSE:

The aim of this study was to determine the extent of post-vaccine seroprotection in a large cohort of persons immunized with a recombinant hepatitis B vaccine in Bulgaria

MATERIALS AND METHODS

A total of 923 serum samples from 412 men and 511 women were tested in two laboratory units (Laboratory of Virology, Military Medical Academy, Sofia, and MDL Cibalab, Sofia) over a two-year period (2018 - 2019). The target group includes those covered by routine childhood immunization against hepatitis B, born in 1992 – 2000, who had received; healthy subjects (cadets, candidates for military service) and patients (hospitalized and outpatients). Their mean age was 23.0±2.7 years (23.01±2.9 years for men and 23.0±2.6 years for women). All immunized persons included in this study were HBsAg negative. Of these, a total of 70 subjects tested negative for antibodies against hepatitis B core antigen (anti-HBc total). Quantitative analysis of anti-HBs antibody levels (mIU/mL) was performed using a commercial DiaSorin LIAISON® anti-HBs II quantitative assay kit on the basis of the chemiluminescence immunoassay (CLIA) technique. The assay has a broad linear range from 5 to 1000 mIU/mL. Anti-HBs concentrations (mIU/mL) were automatically calculated by the analyzer. The results were categorized into three groups according to the reported anti-HBs levels and the threshold values of the assay. Samples with anti-HBs concentrations of < 9 mIU/mL were defined as negative, and those with anti-HBs concentrations of e" 11 mIU/mL as positive, respectively. Samples with anti-HBs concentrations between 9 and 11 mIU/mL were classified as equivocal.

Statistical analysis. Numerical variables were reported as mean values with standard deviation (sd), while categorical ones as absolute numbers and proportions as well as 95% confidence interval (CI). The mean values of the two groups were compared by the independent samples t-test. Correlations between categorical variables were assessed via Pearson's chi-squared test, and correlations between quantitative variables via Pearson –s *r* correlation coefficient. Binary logistic regression analysis was performed to assess for binary output (being positive or equivocal vs negative anti-HBs). Results were considered statistically significant at the p<0.05 level. Statistical analysis was performed with SPSS, version 22.0.

RESULTS

In the study period (2018-2019), of a total of 923 subjects immunized with a recombinant hepatitis B vaccine, 44.6% were male (95% CI: 41.4% - 47.9%; n=412) and 55.4% female (95% CI 52.1% - 58.6%; n=511). The mean age was 23 years (sd 2.7); it was similar for both sexes (mean 23.01, sd 2.9 years in men and mean 23.0, sd 2.6 years in women, p>0.05). The target group was subdivided into nine subgroups depending on the year of birth. The largest subgroup was that of the subjects born in 1992 (n=139), while the smallest one was in 1993 (n=80), but the significance was not reached p>0.05 (Table 1).

Table 1. Distribution of the subjects tested for anti-HBs according to year of birth.

Year of birth	n	%	95% CI
1992	139	15.1	12.8-17.5
1993	80	8.7	6.9-10.7
1994	105	11.4	9.4-13.6
1995	88	9.5	7.7-11.6
1996	95	10.3	8.4-12.4
1997	86	9.3	7.5-11.4
1998	110	11.9	9.9-14.2
1999	105	11.4	9.4-13.6
2000	115	12.5	10.4-14.8
Total	923	100	

The sex distribution of the tested subjects varied significantly between years (p=0.023). Male subjects prevailed in the subgroup born in 2000 (54.8%) but accounted for the lowest share in the 1916 subgroup (33.7%). Conversely, female subjects prevailed in the 1996 cohort (66.3%) but had the lowest share among the subgroup of subjects born in 2000 (45.2%) (Table 2).

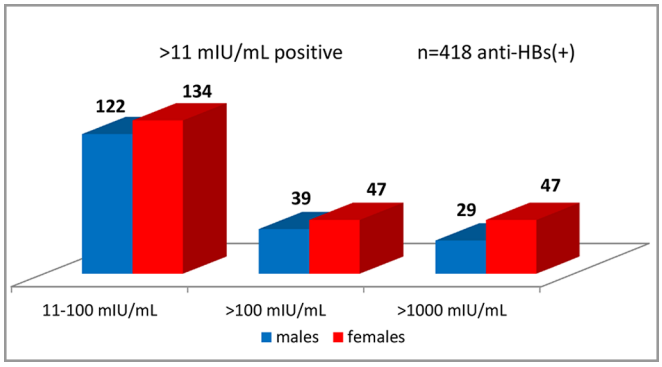
Table 2. Sex distribution of the immunized subjects according to their year of birth.

Year of birth	Gender			
	Male		Female	
	n	%	n	%
1992	71	51.1%	68	48.9%
1993	43	53.8%	37	46.3%
1994	46	43.8%	59	56.2%
1995	33	37.5%	55	62.5%
1996	32	33.7%	63	66.3%
1997	36	41.9%	50	58.1%
1998	46	41.8%	64	58.2%
1999	42	40.0%	63	60.0%
2000	63	54.8%	52	45.2%

The results showed a prevalence of immunized individuals with non-detectable anti-HBs levels of < 9 mIU/mL (negative result), respectively 51.3% (95% CI: 48.1% - 54.6%). The group of subjects with anti -HBs values in the range of 9-11 mIU/mL (equivocal result) accounted for 3.4% (95% CI: 2.2% - 4.2%). The subjects with protective anti-HBs titer of >11 mIU/mL (positive result) were 45.3% (95% CI: 42.0% - 48.6%). There was a similar sex distribution in the cohort of immunized subjects with non-detectable anti-HBs levels (43% male and 57% female) and protective anti-HBs levels (46% males and 54% females) (p>0.05).

The immunized subjects who were anti-HBs positive (190 men and 228 women) were grouped into three subgroups according to the level of seroprotection (Fig.1). The difference in the protective anti-HBs titer between the two sexes was non-significant (p>0.05).

Fig. 1. Sex distribution of the anti-HBs positive subjects (n=418) according to level of seroprotection.



The majority of immunized subjects (n=256) showed protective anti-HBs levels of 11 - 100 mIU/mL. Anti-HBs levels of > 100 mIU/mL were found in 86 subjects, whereas the protective anti-HBs titer was very high (>1000 mIU/mL) in 76 of the tested subjects. The variations in the anti-HBs levels were independent of the sex or year of birth. There was no correlation between sex and the distribution of anti-HBs positive subjects in the protective titer subgroups (p>0.05)

Binary logistic regression analysis was performed to assess whether the year of birth could be considered a determining factor for the distribution of samples among the positive or equivocal vs. negative anti-HBs groups (Table 3). The year of birth was a significant factor in the negative group (p=0.040) with OR 1.051 (95% CI 1.002-1.103), i.e. for every +1year increment in the year of birth, the probability of being in the positive or equivocal groups increased by 5.1% (between 0.2 and 10.3%). In addition, there was a weak negative correlation between the year of birth and the anti-HBs titer (p<0.001, Pearson's r = - 0.351).

Table 3. Distribution of immunized subjects (n=923) according to year of birth and anti-HBs results.

Year of birth	anti-HBs result		
	negative	equivocal	positive
	n	n	n
1992	78	3	58
1993	52	6	22
1994	57	3	45
1995	42	2	44
1996	40	2	53
1997	37	2	47
1998	57	3	50
1999	62	4	39
2000	49	6	60

DISCUSSION

Vaccine prophylaxis has proved to be the most effective measure to prevent HBV infection and its complications. Bulgaria was one of the first countries to include the hepatitis B vaccine in its immunization calendar. There is a well-established primary vaccination schedule with three intramuscular doses (0-1-6 months), with the first dose administered to healthy newborns at the 24th hour after birth. The immune response is assessed by quantitative analysis of anti-HBs levels, which are a marker of immunity.

All subjects included in this study (n=923) received routine childhood hepatitis B immunization. The observed variations in the levels of anti-HBs antibodies in the immunized subjects were independent of sex and year of birth. In 45.3% of the study cohort, there was evidence of existing seroprotection at varying levels (from 11 to >1000 mIU/mL). Our data are similar to those reported by other authors. A study of 141 children in Bulgaria found a post-vaccination protective titer in 67.4% of subjects [11]. The higher percentage of positive results for anti-HBs was most likely due to the fact that the vaccinated individuals studied were aged 5-17 years, whereas in our study, the mean age of the studied cohort was 23.0±2.7 years. Clinical observations have shown that higher vaccine-induced antibody levels are associated with proportionally better post-vaccine protection [12]. A meta-analysis has suggested that the protection provided by the hepatitis B vaccine persists for at least two decades in the majority of vaccinated individuals [13]. Mathematical models indicate that the persistence of anti-HBs levels over time is a function of the achieved peak titers and the time after immunization. Studies have shown

that the decline in anti-HBs levels appears to be proportional to the titer initially acquired [14]. Immunized persons with a very good response to the vaccine (Ã100mIU/mL) had a lower risk of lo routine HBV immunization the anti-HBs at year 15 (76.5%) and year 18 (66.7%) after immunization [15]. The observed decline in seroprotection rate over time is considered a natural process, with the lowest rate in young people in the age range of 20-27 years (12.8%) [16]. In this study, 54.7% of immunized individuals had undetectable levels of anti-HBs (negative or equivocal result).

Observations have shown that post-vaccination immunity is maintained for a long time after the anti-HBs titer falls below the threshold of protection, owing to immune memory. In cases of undetectable anti-HBs levels, administration of a booster dose of vaccine may induce an anamnestic immune response. There may be various reasons for the lack of an adequate immune response to the vaccine, such as incomplete vaccination schedule, improper choice of injection site, improper storage of the vaccine, immunosuppression, obesity, impaired Th lymphocyte response, specific genetic haplotypes (different HLA DR alleles), and variants of the surface antigen [17].

Universal immunization programs have changed the epidemiological profile of HBV infection, leading to a buildup of an immune population at a young age. This is particularly important as adolescence is considered a high-risk period for acquiring HBV infection due to certain behaviors (high sexual activity and unprotected sex, drug use, tattooing). Key indicators of the positive impact of hepatitis B vaccination include a reduction in sources of infection, a reduction in acute and fulminant hepatitis B cases, a reduction in HBV-related mortality due to liver cirrhosis and HCC, and a decrease in HBsAg seroprevalence.

Strengths and limitations of the study

Our study included a relatively large number of subjects who had received the recombinant hepatitis B vaccine (923 in total). The selection criteria were solely the vaccination status and year of birth. The individuals from the target group were born during the period of mandatory hepatitis B immunization (1992-2000), which is still in force in our country. Our results are similar to those reported by other authors, confirming their significance. Our study has some limitations. In Bulgaria, antibody titer testing after completion of the primary immunization course is not a routine practice; therefore, we had no access to data about the baseline concentrations of anti-HBs. A study of 96 dental students aged 20-25 years reported that only 13.9% of participants had had their anti-HBs titer checked [18].

About 5% of immunocompetent individuals do not respond to the vaccine and are conventionally referred to as "nonresponders" [19]. In these individuals, in cases where revaccination is recommended, the HBsAg status needs to be determined prior to vaccination to rule out existing active HBV infection. In our study, active HBV infection was ruled out because all subjects tested negative for HBsAg. Furthermore, undetectable levels of anti-HBs do not necessarily imply loss of immunity due to the presence of immune memory. We could not assess the anamnestic immune response because the administration of a booster dose of vaccine is not mandatory according to our current regulations. Follow-up is advisable but is voluntary. Considerations also include cost-effectiveness. A paradigm shift in the vaccination protocol would help to better control HBV infection. It has been suggested that the half-life of antibodies is much longer when a repeated vaccine series is administered [20].

Another limitation of our study is that the distribution of immunized individuals by year of birth did not correspond to their actual proportions in the general population. Since 1989, there has been a serious decline in birth rates, which has continued in recent years [21]. However, our analysis did not demonstrate a significant difference in the distribution of the tested subjects by their year of birth, which may be due to the small subsample size.

CONCLUSIONS

Mass immunization in childhood and achieving high vaccine coverage play a key role in controlling hepatitis B and preventing its serious public health consequences. Worldwide evidence suggests that routine immunization of all healthy newborns against HBV has reduced the prevalence of infection in children, adolescents and young adults. In our study, we detected varying levels of anti-HBs antibodies in the immunized subjects, independent of sex and year of birth. On average, 23 years after mandatory childhood hepatitis B immunization, we found a protective anti-HBs antibody titer in 45.3% of vaccinated individuals. We believe that monitoring the anti-HBs levels is important to assess the level of seroprotection. Recommendations for immune response monitoring are discussed, as well as the need for booster vaccine administration in immunocompromized individuals and individuals with ongoing risk behaviors.

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