Family Medicine & Primary Care Review 2024; 26(2): 246–250, https://doi.org/10.5114/fmpcr.2024.139037

ORIGINAL PAPERS

ISSN 1734-3402, eISSN 2449-8580

Assessment of anti-HBs level in serum of future healthcare workers in terms of hepatitis B infection prevention

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary **Background.** The Hepatitis B Virus (HBV) infection continues to be a global health problem despite existing vaccinations. Healthcare Workers (HCWs) worldwide face serious risks from infection due to their high contact with infectious material. It is estimated that post-vaccination seroprotection is lifelong or at least lasts 20 years by providing circulating antibodies to the surface HBV antigen (HBsAg).

Objectives. The aim of the study was to examine the level of anti-HBs among a group of future HCWs.

Material and methods. The study group consisted of 90 medical students. Blood samples were collected and anti-HBs concentrations were measured by diagnostic test (ELISA kit, Antisurase B-96 TMBII).

Results. All patients declared they received a mandatory vaccination as a child. A booster dose in the last 10 years was taken by 11.1%. 90% did not know if someone from their surroundings/families suffered from HBV infection. The total average antibody level was 24.93 mIU/mL (range: < 6.76–309.438). There were 56 (62.2%) samples with a very low concentration (< 10 ml mIU/mL), judged as a non-reactive value, and the remaining (34 ppl/37.8%) with a reactive value (range: 10.957–309.438 mIU/mL). Moreover, the obtained concentrations were found to correlate with the time elapsed since the last vaccination, with the result showing a trend towards significance (*p = 0.0571).

Conclusions. The findings suggest that post-vaccination immunity declines over time in most of cases. In case of potential infection, people with a higher risk, like HCWs should have elevated anti-HBs levels for safety reasons. For better prevention and risk stratification in the group of HCWs, periodic examination should be provided.

Key words: vaccinations, hepatitis B, health personnel, medical students.

Zawiła L, Kozioł MM. Assessment of anti-HBs level in serum of future healthcare workers in terms of hepatitis B infection prevention. *Fam Med Prim Care Rev* 2024; 26(2): 246–250, doi: https://doi.org/10.5114/fmpcr.2024.139037.

Background

Hepatitis B virus (HBV – hepatitis B virus) is a well-known pathogen able to cause acute or chronic hepatitis, which can progress to cirrhosis and the development of hepatocellular carcinoma (HCC). In 2011, the World Health Organization (WHO) recognized hepatitis B as a global health problem [1]. Despite mandatory immunization programs among newborns in many countries, as well as initiation of other prevention strategies and access to treatment, an estimated 296 million people were living with chronic infection in 2019. Among this group, only 10.5% were aware of their infection, and 22% were undergoing treatment. The large number of deaths, amounting to 820,000 in 2019, indicates that the worldwide problem still exists [2]. In May 2016, the World Health Assembly endorsed the Global Health Sector Strategy (GHSS), which aims to reduce new HBV and HCV infections by 90% and mortality from them by 65% by 2030 [3]. Considering this, both specific and non-specific infection prevention methods play a key role in the strategy.

Routs of transmission of HBV infection include percutaneous or transmucosal exposure to infected blood or body fluids, i.e. saliva, semen, cervical secretions. The main are vertical (from infected mother to child), horizontal (from carrier to healthy person), sexual, and extraintestinal transmissions [4–6]. For non-specific prevention methods, we can include avoiding risky sexual intercourse or intravenous drug use, as well as maintaining the required sanitary conditions for injection procedures and high safety control of blood used for transfusions [7]. The WHO has placed great emphasis on education of the public, especially those in at-risk groups, about the threats of HBV infection. More than 80% of patients with chronic infection are unaware of their disease, so increased diagnostics, including the introduction of self-testing, have become a global goal [8].

Vaccination is currently the most effective method of preventing HBV infection. The first commercially available HBV vaccine was a plasma-derived vaccine developed in 1982, and nowadays a recombinant vaccine is used (3 doses required) [9, 10]. Following through the entire recommended vaccination schedule ensures protective antibody titers in more than 96% of cases in newborns, children, and adolescents, as well as in more than 90% of cases in adults [10]. Post-vaccination immunity against HBV is considered as long-lasting, even if antibody titers fall below the cutoff. Studies indicate that some people who have lost the recommended antibody titers in their teenage years do not respond to a booster dose of vaccination, which may indicate low protection against infection [11–13]. The WHO does not recommend extra booster doses as standard but suggests it for people at risk [10]. Healthcare workers (HCWs) are particularly at risk of infection due to very frequent contact with infectious material [14], and HBV infection is a serious occupational hazard for HCWs worldwide. The aim of this study was to evaluate HBV

seroprotection by checking the concentration of anti-HBs in the serum of medical students, in terms of the maintenance of post-vaccination immunity.

Material and methods

The study group consisted of 90 patients – medical students (first and second year): 65 women (72.2%) and 25 men (27.8%), aged 20–25 years (on average 21.31 years).

The students who applied to the Laboratory of Microbiology in the Chair and Department of Medical Microbiology at the Medical University of Lublin for screening tests were initially interviewed with a questionnaire about HBV infection and their hepatitis B vaccination. Proper written informed consent was obtained from all subjects to use the obtained analysis results for scientific purposes.

Blood samples for laboratory analysis were collected, and the serum was separated by centrifugation. An enzyme-linked immunosorbent assay, ELISA (commercial ANTISURASE B-96 II (TMB) kit; GBC/General Biologicals Corporation, Taiwan), was performed to detect the concentration of anti-HBs. The kit was used according to the manufacturer's instructions. Anti-HBs interpretation ranges: < 10 mIU/mL indicated as *non-reactive/negative*; ≥ 10 mIU/mL indicated as a *reactive/positive*.

The obtained laboratory results and data from the survey underwent statistical analysis, and the occurring correlations were checked. The Statistica v.12.5 (StatSoft) and MS Excel 2010 (Microsoft) programs were used to collect/enter data and support the above-mentioned analyses.

Ethical consideration

In the study, laboratory data from those who reported to the laboratory for general testing was used. Written informed consent was obtained from the patients to use the diagnostic results for scientific purposes.

Results

A group of 90 young medical students was analyzed (11 students in the first year and 79 in the second year). In the study group, 41% declared that someone from their relatives works in health care; 83.3% know what kind of genetic material the HBV virus contains and know the potential routes of pathogen transmission (through the blood 100%; by sexual contact: semen – 81.1%/cervical secretion – 66.7%; contaminated medical equipment – 62.2%; acupuncture/tattooing – 77.8%). The majority of respondents do not believe that contact with saliva and living with an infected person contribute to the spread of the virus (85.6% and 92.2%, respectively).

Respondents declared that HBV vaccination is mandatory (86 ppl/95.6%). According to the collected data, 74 subjects/82.2% ($p = 0.7262$) received the vaccination as a child, 13 subjects/14.4% said they had received the vaccination but did not remember when ($p = 0.5779$), and only 3 subjects/3.3% did not know if they had been vaccinated (*$p = 0.0416$).

A booster dose in the last 10 years was taken by 10 ppl/11.1%, and 33 ppl/36.7% did not remember. The remainder indicated that they had not had additional immunization – 47 ppl/52.2% ($p = 0.4565$). In the study group, 70 ppl/77.8% were aware that HBV immunization provides immunity against HDV, which is a dependent virus ($p = 0.4608$). At the same time, the entire study group did not know what immunity they had against HBV and had never received passive immunization before. The majority (81 ppl/90%) had no knowledge about HBV infection among their relatives. Medical students described their risk of potential infection at different levels: as very high – 24 ppl/26.7%; high – 61 ppl/67.8%; medium – 3 ppl/3.3%; and even low – 2 ppl/2.2% ($p = 0.6305$). Only 8 ppl/8.9% donate blood regularly to a blood center ($p = 0.7331$).

Seroprevalence against HBV in the group was at a variable level (low or medium). The analyses showed that 56 subjects (62.2%) had an anti-HBs concentration < 10.00 mIU/mL, and according to the test interpretation, this is assessed as non-reactive and should be considered negative. Of this group, 49 people had very low immunoglobulin levels of < 6.76 mIU/mL, which is below the kit cut-off/analytical sensitivity of the test. Reactive/positive results were observed in 34 people (37.8%), of which highly reactive > 100 mIU/mL – was observed in 5 people. However, the manufacturer of the diagnostic test emphasizes that a low-positive result is not proof that a patient is completely safe from hepatitis B virus infection, and the evaluation of the samples is in a line with CLSI recommendations and is based on the WHO international standard for anti-HBs as an indicator of an immune status. Statistical analysis showed that the obtained antibody concentrations correlated with the time that had passed since the last vaccination. The result showed a trend towards significance (*$p = 0.0571$).

The average antibody titer of all subjects was 24.93 mIU/mL (range: < 6.76–309.43; SD 51.90). Detailed results are presented in Table 1.

Data from the survey was collected and analyzed (Chi-squared test) according to the interpretations of the test result (*non-reactive* vs *reactive*) and is presented in Table 2.

Discussion

Active immunoprophylaxis is one of the most efficient ways to fight infectious diseases. In case of hepatitis B infection prevention, recombinant vaccines are used nowadays. They produce an immune response for the viral s antigen, which results

Table 1. Laboratory findings of anti-HBs and interpretation provided in the ANTISURASE B-96 II (TMB) kit (GBC/General Biologicals Corporation, Taiwan)

Anti-HBs*			
***n* = 90**	**< 10 mIU/mL *non-reactive/negative***		**≥ 10 mIU/mL *reactive/positive***
	< 6.76 mIU/mL	6.76–9.99 mIU/mL	
	49 (54.4%)	7 (7.8%)	**34 (37.8%)**
	56 (62.2%)		
Antibody concentration mIU/mL	"6.76 mIU/mL" values assumed in the statistical calculations	av. **8.17** (6.96–8.96) [SD 0.81]	av. **54.56** (10.95–309.43) [SD 76.23]
	av. **6.83** (< 6.76-8.86) [SD 0.54]		

SD – standard deviation. * Test manufacturer's comments: a low-positive result in the test is not proof that the patient is completely safe from hepatitis B virus infection.

Table 2. Interpretation of the result (*non-reactive* vs *reactive*) depending on the selected categorized variables collected in the survey

Study group		Interpretation of anti-HBs result		*p*
		non-reactive	*reactive*	
Gender				0.3184
female		43 (66.2%)	22 (33.8%)	
male		13 (52%)	12 (48%)	
Study year				0.6635
I		8 (72.7%)	3 (27.3%)	
II		48 (60.8%)	31 (39.2%)	
Do any of your relatives work in health care?				
yes		24 (64.9%)	13 (35.1%)	
no		32 (60.4%)	21 (39.6%)	
Vaccination for HBV	"I was vaccinated on the first *day* of life"	46 (62.2%)	28 (37.8%)	0.7956
	"I was vaccinated, but I don't remember"	9 (69.2%)	4 (30.8%)	0.7993
	"I do not know if I was vaccinated"	1 (33.3%)	2 (66.7%)	0.6570
	Vaccination is …			0.9906
	mandatory	53 (61.6%)	33 (38.4%)	
	recommended	3 (75%)	1 (25%)	
	Does the vaccine for HBV provide a protection for other viral infection?			0.7039
	yes	42 (60%)	28 (40%)	
	no	10 (71.4%)	4 (28.6%)	
	Have you been vaccinated with a booster dose in the last 10 years?			0.6215
	yes	5 (50%)	5 (50%)	
	no	31 (66%)	16 (34%)	
	I do not know	20 (60.6%)	13 (39.4%)	
Being a blood donor				0.7151
yes		5 (62.5%)	3 (37.5%)	
no		51 (62.2%)	31 (37.8%)	

in the production of so-called anti-HBs. Artificial active immunization against the hepatitis B virus has been an obligatory part of infant health care in Poland since 1996 and is also recommended for previously unimmunized persons. A high-risk group of infection includes people undergoing invasive procedures in a hospital, travelers, people infected with HCV, people whose partners and household members are infected with HBV, as well as healthcare professionals and students of medical schools [10, 15]. According to recommendations, this last group should recurrently check their anti-HBs levels, and if antibody titers drops < 10 mIU/mL, they should consider an additional dose of the vaccine [16].

Previous research assessing post-vaccination immunity among a group of young people showed a significant loss of antibody titers over the years [17–19]. Our results are consistent with these reports, while the percentage of non-reactive people compared to most studies is higher (62.2%). Statistical analysis showed that the obtained concentrations correlated with the number of years since the last vaccination. The result showed a trend towards significance (*p = 0.0571). Researchers Pileggi et al. reported antibody titers < 10 mIU/ml in over 25% of the subjects, and Al Ghamdi et al. in about 41% [18, 19]. This difference may result mostly from our study group quantity (90 people), but it should also be taken into account that vaccination programs vary between countries. Tosun et al. showed a decrease in the protective titer of antibodies in 45.7% of children just 9 years after vaccination [17].

Healthcare workers are a group at high risk of HBV infection, while young medical students are at even greater risk due to the lack of experience in performing certain procedures and handling infectious material [20]. According to AASLD (American Association for the Study of Liver Disease) recommendations, these individuals should be tested for a response to vaccination 1 to 2 months after the last dose of the vaccine [21]. This procedure is not possible for individuals who were vaccinated as infants and did not receive a booster dose. For this reason, screening tests are also recommended. Unfortunately, data gathered by Vivian Efua et al. among HCA in Ghana shows that only 21.3% of the respondents complied with the recommendations for assessing the serological status [22]. Such little interest in one's own immunity to the virus may be due to the WHO's position that there is no need to take booster doses.

The results of studies assessing the long-term persistence of post-vaccination immunity showed a mainly positive response of the subjects to the challenge/booster dose. Evaluating seroprotection after 22 years of primary vaccination, McMahon et al. achieved a response to a booster dose among 81% of the subjects, whose antibody titers were initially < 10 mIU/ml [23]. A continuation of this study was conducted by Bruce et al. [12]. Of those who were not vaccinated after 22 years and their antibody concentration dropped below 10 mIU/ml, 88% responded to the booster dose. Additionally, data collected by Varshochi et al. among healthcare workers indicated that after the booster dose, the percentage of non-reactive people decreased from 9.4% to 2.1% [24]. However, in all of these studies, there was a percentage of subjects who did not respond to the booster dose. Our results also showed this dependency. We asked respondents whether they had received a booster dose in recent years. Those who declared receiving a booster dose in the last 10 years (10 people/11.1%) turned out to have a different immune response: *reactive*/5 people, and *non-reactive*/5 people. Lack of response may indicate the loss of a strong immunity.

Limitations of the study

The limitation of our own research was primarily the small size of the study group. Due to the lack of access to medical records, we had to base our conclusions only on the information obtained in the survey, which may have differed from the true situation. We also could not independently administer a booster dose to all non-reactive subjects and assess their appropriate response.

So far, the mechanism of immunity to HBV has not been fully resolved. For this reason, it cannot be clearly stated whether people whose antibody titer is < 10 mIU/ml are not immune and whether the lack of response to a booster dose is equivalent to a loss of immunity. For increased safety, it is expected that HCW belonging to the group at high risk of infection will show increased titers of anti-HBs. Being regularly screened and taking booster doses will significantly help to control seroprotection.

Conclusions

Hepatitis B infection can manifest as a short and acute disease or, in some patients, as a chronic disease progressing into cirrhosis and HCC (hepatocellular carcinoma). Introduction of preventive vaccination programs in many countries significantly decreased the spread of the infection. Although postvaccination seroprotection is considered to be lifelong, scientific reports suggest a relevant loss of antibodies several years after vaccination. Self-provided tests confirm this observation, where 62.2% of patients had low anti-HBs levels assessed as non-reactive. Obtained antibody concentrations correlated with the time that had passed since the last vaccination.

This demonstrates a significant reduction in immunoprotection over time. The study group consisted of future healthcare workers, who are in a high-risk group of infection due to regular contact with infectious material. Due to the obtained results, it seems appropriate to control anti-HBs levels in high-risk groups of patients and, for safety reasons, maintain them above the recommended levels by taking a booster dose if needed.

Source of funding: This work was funded from the author's own resources.
Conflicts of interest: The authors declare no conflicts of interest.

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Figures: 0
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Received: 21.11.2023
Reviewed: 27.11.2023
Accepted: 02.12.2023

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