IMMUNE RESPONSE AND IMMUNOLOGIC MEMORY IN MEDICAL PERSONNEL VACCINATED WITH HEPATITIS B VACCINE

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ABSTRACT
The occupation-related nature of Hepatitis B viral infection in medical personnel has been well documented in a lot of studies. The only reliable way of prevention of this infection is immunisation with hepatitis B vaccine.

AIM: To follow-up the primary immune response after immunisation with recombinant vaccine and its duration in adult immunocompetent subjects.

MATERIALS AND METHODS: One hundred sixty-five health-care workers working at St. George University Hospital, Plovdiv in 2009/2010 were included in the study and allocated to two groups. Group 1 (N1 = 70) was followed up for the primary immune response after immunization; group 2 (N2 = 95) was with documented immunization in 1998/1999 (n = 81) and in 1994/1995 (n = 14). Tests based on ELISA for quantitative determination of anti-HBs in mIU/ml were used. The measurement were performed at the National Reference Laboratory of Viral Hepatitis at the NCIPD, Sofia. Descriptive statistics, non-parametric and parametric tests, qualitative correlation were used to analyse data.

RESULTS: Group 1 mean age was 40.3 ± 2.6 years; anti-HBs concentration of ≥ 10 mIU/ml was found in 92.8%. No association between the immune response and the commonly involved factors such as gender, age, overweight, smoking, etc., was found. In group 2, anti-HBs concentration of ≥ 10 mIU/ml was found in 77.9%: it was in 75.3% in those immunized 10 years before, and in 92.9% in those immunized 15 years before (t = 0.24, p > 0.05). A booster dose of the vaccine was received by 15/21 subjects from group 2 (those immunized 10 years before that) with anti-HBs < 10 mIU/ml. After the booster, 9/15 produced anti-HBs in protective concentrations (anamnestic immune response). The actual level of seroprotection among the immunized more than 10 years ago was 92%.

CONCLUSION: This study and the documentation of the primary postvaccinal immunity in high-risk medical personnel will help specify if additional hepatitis B vaccine shots are needed.

Key words: hepatitis B vaccine, medical personnel, seroprotection, anti-HBs antibodies

INTRODUCTION
The occupation-related nature of Hepatitis B viral infection in medical personnel has been well documented in many studies.1-5 Getting infected from a patient had been common before the introduction of specific immunoprophylaxis. A study in the USA among medical staff in the prevaccinal period, conducted by the Center for Disease Control and Prevention (CDC, Atlanta), reported nearly 12000 cases per year of hospital acquired infection with hepatitis B virus (HBV) resulting from exposure to infectious blood at work.6 The only reliable way of preventing this type of infection and its related complications such as liver cirrhosis and hepatocellular carcinoma is immunization with hepatitis B vaccine.

CDC does not recommend routine screening of HbsAg before immunization, as well as routine study of the postvaccinal response (anti-HBs antibodies).7 In cases of vaccination after the age of 50, in presence of obesity, immune deficiency and high risk of contact with blood and/or other
body fluids, follow-up of the primary seroconversion after vaccination is advisable.3,7-9

AIM
The aim of the study was to follow-up the primary immune response after immunization with recombinant hepatitis B vaccine and its duration in adult immunocompetent subjects (medical personnel), so that recommendations for improving prevention can be made.

MATERIALS AND METHODS
The study included 165 health-care workers at St. George University Hospital, Plovdiv between 2009 and 2010 with no medical history of hepatitis infection. These subjects were divided into two groups: in group 1 (N1 = 70) we followed up the primary immune response (1-3 months) after immunization with Hepavax Gene, Berna Biotech Italia S.r.l, Italy, 20μg HbsAg /1 ml, i.m. applied in m. deltoideus, complying with a scheme of 3 doses (0-1 month-6 month); Group 2 (N2 = 95) consisted of individuals with documented immunization with recombinant hepatitis B vaccine in 1998/1999 (n = 81) and in 1994/1995 (n = 14).

Written informed consent was obtained from all participants. The study did not include subjects that had received additional doses of the vaccine. The attached questionnaire included information about demographic characteristics such as age, sex, job position and employer information, immunization scheme, history of chronic diseases, immune system impairments, smoking, and body mass; and the resultant parameters – hepatitis infection in the past, concentration of anti-HBs antibodies.

Tests based on ELISA -Antisurase B-96 (TMB) General Biological Corporation, Taiwan and Monolisa Anti-HBs Plus, France, were used. The quantity of anti-HBs IgG was determined in mIU/ml at these intervals: 10–50 mIU/ml, 50–100 mIU/ml and > 100 mIU/ml. Determination of anti-HBs was performed in the National Reference Laboratory of Viral Hepatitis at the National Center for Infectious and Parasitic Diseases (NCIPD) – Sofia. According to the standard response criteria, we have a positive immune response after immunization with recombinant hepatitis B vaccine if anti-HBs ≥ 10 mIU/ml. Individuals with < 10 mIU/ml were defined as non-responders, those with values of 10-50 mIU/ml were classified as “weak responders”, and those with values of 50-100 mIU/ml and > 100 mIU/ml as presenting with “strong” and “very strong” immune response, respectively.

Data were analyzed using the following statistical analyses: descriptive statistics, non-parametric analysis χ² (Pearson chi-square test), Fisher’s Exact test, Student t-test. Quantitative parameters are presented as mean ± SD. Level of significance for the null hypothesis was accepted at p < 0.05. Data were analyzed with statistical software SPSS v.11. Graphical presentation of the results is performed with Microsoft EXCEL v.2007.

RESULTS

 Primary immune response
The mean age of the study population was 40.3 ± 2.6 years. The graphical view of age distribution is presented in Fig. 1. The relative percentages of the women is 81.4% with male to female ratio of 1:4.4.

The relative percentage of medical workers that produced protective anti-HBs antibodies was high (92.8% with Sp = 3%). The relative percentage of non-responders and individuals with “weak” and “strong” immune response was almost identical (Fig. 2).

Eighty percent of the investigated persons had “very strong” vaccinal response (anti-HBs > 100 mIU/ml), which is typical for clinically healthy individuals, in contrast to the postvaccinal response in hemodialysis patients in whom antibody production in this range was observed in only 48.9% of the patients.10 N. Gacheva et al., studying the primary postvaccinal response in medical personnel, found similar results – seroconversion (presence of anti-NBs antibodies) was found in 95.5% of the immunized subjects, the anti-HBs concentration being greater than 100 mIU/ml in 86.4%.11

As seen from the age-distribution of postvaccinal antibody quantity (Fig. 3), all immunized subject in the 20–29-year-olds reacted by producing protective titer of anti-HBs > 10 mIU/ml, while in the group age ≥ 50 years the relative percentage of non-responders was as much as 12.5%.

Three of the non-responders received another (fourth) booster dose of the vaccine. Of these 1 subject responded with concentration of anti-HBs > 100 mIU/ml which in creased the relative percentage of protected people to 94.3%. According to the established rules, individuals who had not reached protective concentration of anti-HBs after the fourth application, accomplished the second immunization series with two more doses or cumulatively (3+3), with follow-up of the serum antibodies 1-2 months after the last application.13 There has been no clear explanation yet in literature why some individuals
need more applications to reach optimal immune response, assuming that non-responders from the first vaccinal series would respond in 30-50% with protective titers after the second series of three applications.\textsuperscript{3,12,13}

To find the reason for unsuccessful vaccination (a 3-dose scheme), it is recommended that subject be investigated for the presence of hepatitis markers (HBsAg, anti-HBc)\textsuperscript{1,3,8}, especially in view of their mean age and their long occupational exposure to blood.

The subjects’ distribution by clinic they work in was as follows: 38.6% worked in surgery divisions of the hospital and all (100%) responded with protective concentration over 10 mIU/ml, while in the group of subjects working in non-surgical clinics presence of anti-HBs antibodies was found in 88.4% (\(p > 0.05\)). According to their professional position primary immune responders were respectively: 100% of the medical doctors, 92.5% of the nurses and 83.3% of the hospital attendants (\(\chi^2 = 1.68, df = 2, p > 0.05\)).

A number of factors like old age, male gender, overweight and obesity, smoking, diseases, leading

![Figure 1. Distribution of medical personnel by age groups in studying the primary immune response (n = 70).](image1)

![Figure 2. Distribution of medical personnel by anti-HBs titer (n = 70).](image2)
Immune Response and Immunologic Memory in Medical Personnel Vaccinated with Hepatitis B Vaccine

to disturbances in immunity, certain HLA haplotypes, etc. are among the commented and investigated reasons associated with weaker postvaccinal immune response.1,3,14-17

We followed-up the effect of 5 factors (age, gender, weight, smoking and immunity-modifying concomitant diseases) on the postvaccinal immune response (Table 1).

The study sample was divided into 2 age groups: 20–49-year-olds and older than 50 years, and into two weight groups (people with normal and people with abnormal, increased weight) according to their personal assessment, documented in their questionnaires.

**Table 1.** Distribution of postvaccinal immune response by sex, age, body weight, smoking, etc.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>anti-HBs &gt; 10 IU/ml</th>
<th>anti-HBs &lt; 10 IU/ml</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>men</td>
<td>12 (92.3)</td>
<td>1 (7.7)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>women</td>
<td>53 (93)</td>
<td>4 (7)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–49 yrs</td>
<td>51 (94.4)</td>
<td>3 (5.6)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>≥ 50 yrs</td>
<td>14 (87.5)</td>
<td>2 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>overweight</td>
<td>14 (87.5)</td>
<td>2 (12.5)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>normal</td>
<td>51 (94.4)</td>
<td>3 (5.6)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25 (86.2)</td>
<td>4 (13.8)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>No</td>
<td>40 (97.6)</td>
<td>1 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Concomitant diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>No</td>
<td>64 (94.1)</td>
<td>4 (5.9)</td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s exact test, two tailed p-value.
DURATION OF THE IMMUNE RESPONSE

The mean age of the second group was 46.1 ± 1.82 years. Graphical presentation of their age distribution is shown in Fig. 4. The relative percentages of females was 81.1% with male to female ratio of 1:4.6.

Despite the expected decrease in the postvaccinal antibodies concentration (anti-HBs) below the borderline, titers of over 10 mIU/ml were found in the majority of subjects (77.9%) (Fig. 5). In the subjects immunized 10 years before this study, seroconversion (presence of anti-HBs antibodies) was found in 75.3%, and in those immunized 15 years before - in 92.9% (t = 0.24, p > 0.05). Many other researchers have found decrease of the antibodies concentration below the detectable threshold in 10-50% of the subjects immunized 10-15 years prior. It is worth noting that the absence of antibodies (anti-HBs < 10 mIU/ml) in previously immunized subjects does not prove absence of protection. There have been studies demonstrating that postvaccinal immunity against hepatitis B is not only humoral, but it has been detected that immunologic memory determined by HBsAg – specific T-lymphocytes persists long time after vaccination in recipients with serum antibodies < 10 mIU/ml. The presence of immunologic memory may be proven indirectly by measurement of the immune response to a booster vaccine dose, accepted as anamnestic immune response (AIR). AIR can be shown in two ways: in subjects with concentration of ≥ 10 IU/ml – 4-fold or greater increase of antibodies quantity and in subjects with concentration < 10 IU/ml - increase up to

![Figure 4. Distribution of medical personnel by age groups in studying postvaccinal immune response duration (n = 95).](image)

![Figure 5. Distribution of medical personnel by anti-HBs titer (n = 95).](image)
and over 10 IU/ml, measured 2 to 4 weeks after the booster dose.\textsuperscript{19}

The algorithm we used was the administration of a booster dose of the vaccine only in individuals with concentration of anti-HBs < 10 IU/ml. Fifteen (71.4\%) out of 21 health-care workers with anti-HBs < 10 mIU/ml continued their participation in the study and received a fourth vaccine application. Nine of them responded positively (with anti-HBs > 10 IU/ml). The real level of seroprotection (LSP) we found was 92\%, calculated by the formula proposed by J. Poorolajal\textsuperscript{19}, in which LSP is the sum of the proportion of protected (PP) and the product of the proportion of non-protected subjects (PNP) and the proportion of non-protected with anamnestic immune response (PAIR):

\[
LSP = \left( \frac{PP}{PP + (PNP \times PAIR)} \right) \times 100 \quad \text{or} \\
LSP = \left[ \frac{74/95 + (21/95 \times 9/14)}{74/95 + (21/95 \times 9/14)} \right] \times 100 = 92\% 
\]

**DISCUSSION**

A fact which cannot remain unnoticed is the relatively late age of primary immunization. This suggests that there is certain weaknesses in the organisation and administration of preexposition immunoprophylaxis in health system units in Bulgaria, as part of the system for prevention of occupational risk of infection with hepatitis B. The right strategy would require that vaccination should be done before admission to medical college/university or beginning work at medical departments (prior to the contact with blood or blood products).\textsuperscript{3}

The evidence in the available literature points to 5-10\% of the adult population that does not respond to standard HBV vaccination\textsuperscript{3,16}, and among medical personnel the share of non-responders may reach considerably higher levels of 5-32\%.\textsuperscript{17} The absence of seroconversion in 7.2\% of the investigated subjects after the first immunologic response may be partially related to the existing compromising factors. On the other hand, the individual immunologic characteristics must be mentioned, because immunity in hepatitis B viral infection (both natural and acquired) is a complex combination of T- and B- cellular mediation, which in certain populations and HLA types is related to unsuccessful vaccination. It is assumed that definitive “non-responders” are subjects that have not been able to produce anti-HBs > 10 IU/ml after two complete immunization series and with negative hepatitis markers, which makes them susceptible to HBV.\textsuperscript{12} Based on expert recommendations, follow-up of the primary immune response in risk medical personnel is necessary, which will specify more precisely the additional doses of the vaccine.\textsuperscript{9,20}

Immunization of medical personnel with hepatitis B vaccine provides a reliable, long-term protection, even after decrease in the titer of anti-HBs < 10 mIU/ml, which is evidenced by the high level of seroprotection in over 90\% of the subject immunized more than 10 years ago.

**CONCLUSIONS**

The present study determines the level of protection after hepatitis B immunization in adult clinically healthy individuals, particularly medical personnel. No correlation was found between the investigated factors (age, sex, body weight, smoking and concomitant immunity disturbance) and the immune response. There is a serious problem, both organisational and financial, in how to make the immunization as early as in medical universities/colleges. All medical personnel, which is at higher risk of exposure to bloodborne pathogens, should have a documented test for post-infection / postvaccinal immunity against hepatitis B (level of protective antibodies anti-HBs > 10 mIU/ml).

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ИММУННЫЙ ОТВЕТ И ИММУННАЯ ПАМЯТЬ У МЕДИЦИНСКОГО ПЕРСОНАЛА, ИММУНИЗИРОВАННОГО ГЕПАТИТОМ Б ВАКЦИНОЙ

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РЕЗЮМЕ

ВВЕДЕНИЕ: Профессиональный характер гепатитов В и С вирусной инфекции у работников здравоохранения документирован в ряде исследований. Единственным надежным способом превентии является иммунизация персонала вакциной.

ЦЕЛЬ: Работа ставит себе цель проследить первичный иммунный ответ после иммунизации рекомбинантной вакциной, а также и его продолжительность у взрослых иммунокомпетентных лиц.

МАТЕРИАЛ И МЕТОДЫ: Обследовано 165 работников здравоохранения (Университетская больница имени «Святого Георгия», г. Пловдив в 2009 – 2010 гг.), разделенных на 2 группы:
• Первая группа (N1 = 70) – прослежен первичный иммунный ответ в результате проведенной иммунизации
• Вторая группа (N2 = 95) – документирована иммунизация в 1998/1999 (n = 81) и иммунизация в 1994/1995 г. (n = 14).

Авторы использовали тесты, базированные на методе ELISA, в целях определения количества anti-HBs в mIU/ml. Исследование проведено в Национальной референциальной лаборатории “Гепатитные вирусы” при НЦЗПБ, София. Применены дескриптивная статистика, непараметрический тест, параметрический тест, качественная корреляция.

РЕЗУЛЬТАТЫ: Средний возраст медицинского персонала первой группы – 40.3 ± 2.6 лет, при чем концентрация anti-HBs ≥ 10 mIU/ml установлена в 92.8% случаев. Ассоциация между иммунным ответом и обычай дискутированными факторами (возраст, наследственность, курение и др.) не установлена.

Во второй группе количество anti-HBs ≥ 10 mIU/ml установлено в 77.9%; среди иммунизированных 10 лет назад в 75.3%, а среди иммунизированных 15 лет назад в 92.9% случаев (t = 0.24, р > 0.05). 15/21 работникам здравоохранения второй группы, иммунизированным 10 лет назад количеством anti-HBs < 10 mIU/ml, проведена бустерная (дополнительная) доза вакцины, после чего 9/15 образовали anti-HBs в протективных концентрациях (анамнестический иммунный ответ). Реальный уровень серопroteкции среди иммунизированных ≥ 10 лет назад составляет 92%.

ЗАКЛЮЧЕНИЕ: Исследование и документирование первичного поствакцинального иммунитета у высококровного медицинского персонала уточняет необходимость в дополнительных приемах гепатит Б вакцины.