Assessment of protective serum anti-HBsAb levels among previously HBV vaccinated medical residents and its relation to duration and doses of vaccine – single center study

Mohamed B Hashem*, Hanan Abd El-Halim¹, Marwa Khairy¹, Ayatallah Amir Nassef², Mahmoud Essam El-Din¹, Eman D El Desouky³.
¹- Endemic Medicine and Hepatology Unit - Faculty of Medicine - Cairo University, Egypt
²- Clinical Pathology Department - Faculty of Medicine - Cairo University, Egypt
³- Epidemiology and Biostatistics - National Cancer Institute - Cairo University, Egypt

ABSTRACT

Background: Hepatitis B virus (HBV) vaccination is mandatory among health care workers due to high risk of exposure to infection. However, the long-term protective effect of HBV vaccine and assessment of HBV vaccination status by measuring HBsAb remain questionable. Aim: assess the protective levels of anti-HBsAb among previously vaccinated medical residents and its relation to duration and doses of vaccine. Methods: Cross section study conducted on 202 residents working in Kasr Al-Aini Medical hospital (single center) HBV vaccinated. Serum HBsAb titer was tested using Elecsys® Anti-HBs II reagent by automated COBAS device system e-601. The residents were classified according to their protective antibody titer into three groups; negative protective titer (<10IU/L), positive low protective titer (10-100 IU/L) and high positive protective titer (>100 IU/L) groups. Results: Only 9.4% had negative protective titer, 35.15% had positive low protective titer and 55.45% had positive high protective titer. Negative protective HBsAb titer was significantly higher in males (15.5%), smokers (26.5%), with incomplete vaccination schedule (26.6%), compulsory vaccinated (53%) and last vaccine dose >10 years (60.7%). Multivariate logistic regression analysis revealed that the duration since last vaccination dose (<10years) and completion of vaccination schedule (≥3doses) are the significant independent factors for HBsAb protective levels with p value <0.001 and 0.012. Conclusions: Protective anti-HBsAb level is related to the number of years elapsed since vaccination and the number of vaccine doses.
protective effect of hepatitis virus vaccine and the need for booster dose vaccination remains unclear. It is estimated that 13–60% of initial responders to HBV vaccine may lose detectable anti-HBs in subsequent years [4].

Studies have demonstrated that the majority of individuals elicit a strong anamnestic response upon exposure to HBV even 10–15 years after primary vaccination in infancy [5]. However, the rate of protection decreases with increasing age from more than 90% in children and infants to 86% in fourth decade to 47% in sixth decade [6].

Routine post-vaccination testing to document anti-HBs seroconversion is unnecessary except in health-care workers, patients on chronic hemodialysis, and other individuals who are at risk for recurrent exposure to hepatitis B [7].

Objectives

The objectives of our study are to assess the protective levels of anti-HBsAb among previously vaccinated medical residents and its relation to duration and doses of vaccine.

Material and methods

Selection of patients

Cross sectional study is conducted on 202 residents working in Kasr El-Aini teaching hospital – faculty of Medicine – Cairo University from various clinical specialties who received hepatitis B vaccination either as a part of the national compulsory vaccination program in Egypt at the age of 2, 4 and 6 months or a noncompulsory vaccination program.

Participants were subjected to fill a written questionnaire including; the age, sex, residence, working specialty, smoking, presence of chronic medial illness, history of blood exposure and needle stick injuries, vaccination status(complete or incomplete vaccination, compulsory or non compulsory vaccination, booster vaccines received) and time received the last vaccine dose.

HBsAb detection

Participants informed written consent and local ethical committee approval were available before starting data collection. With respect to participants’ confidentiality, participants were represented in the study by code numbers and not by their names with all personal data concealed. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

A 5ml of blood is withdrawn from each participant using a 10cc needle syringe and added to an empty non heparinized tubes. The collected samples are centrifuged the same day of sample collection. The centrifugation cycle is 10 minutes at 2200-2500 RPM. The separated serum is withdrawn using a pipette to be added in eppendorf tubes and stored at temperature of -20°C.

Anti-Hepatitis B surface antigen (HBsAb) is detected using Elecsys® Anti-HBs II reagent by automated COBAS device system e-601.

Participants’ classification

Participants were classified according to their protective antibody titer into three groups: negative protective titer (< 10IU/L), positive low protective titer (10-100 IU/L) and high positive protective titer (> 100 IU/L) groups [8].

Statistical Analysis

Data management and statistical analysis are performed using the Statistical Package for Social Sciences (SPSS) version 24. Numerical data is summarized using means and standard deviations. Data is explored for normality using Kolmogorov-Smirnov test and Shapiro-Wilk test. Categorical data is summarized as percentages.

Comparisons between the 2 groups with respect to normally distributed numeric variables are done using the t-test. Non normally distributed numeric variables are compared by Mann-Whitney test. For categorical variables, differences are analyzed with χ2 (chi square) test and Fisher’s exact test when appropriate. P value was considered significant if <0.05.

Spearman rho correlation is used to assess linear correlation between number of vaccine doses and HBsab titer. Stepwise logistic regression was done to give adjusted odds ratio and measure magnitude of the effect of different factors for being not protected.

Results

Baseline characteristics of the studied population

The age of the studied resident was ranging between 25 to 30 years with the mean of 26.7 years. 52% of the studied residents were females (105 residents), most of the studied population 87.6% was living in urban areas (177 residents) and 34 residents (16.8%) were smokers. Regarding history of direct exposure to blood spill 99.5% (201 residents) of the studied residents have positive
history and 82.7% (167 residents) had history of one or more needle stick injury.

Concerning vaccination history 65.8% (133 residents) were compulsory vaccinated and 34.2% (69 residents) were noncompulsory vaccinated. Of the noncompulsory vaccinated group only 34.78% (24 residents) completed their vaccination schedule.

20.8% (42 residents) received full vaccine series, 8.4% (17 residents) received full vaccine series and an additional booster dose, 10.9% (22 residents) received full vaccine series and 2 booster doses and 37.6% (76 residents) received full vaccine series and 3 booster doses of vaccine as presented in table (1). Regarding the duration of the last vaccination dose; 86% of the studied population (174 residents) received the last dose within the last 10 years (< 10 years) and 14% (28 residents) received the vaccine for a duration more than or equal to 10 years (≥ 10 years).

**Interpretation of HBsAb titer and its relation to baseline characteristics and vaccination status of the studied population**

Negative titer (< 10IU/l) was found in 9.4% of the studied population (19 residents) and positive titer was in 90.6% (183 residents). Positive low protection titer (10–100 IU/l) was detected in 35% (71 residents) and positive high protection titer (>100 IU/l) was achieved in 55.4% (112 residents).

In relation to baseline characteristics of the studied population negative protective HBsAb titer was statistically significantly higher among male residents (15.5%), smokers (26.5%) with p value <0.001 and <0.001 respectively. No significant relation was noticed between the antibodies protective titer and the residence of the history of blood spill exposure and needle stick injuries as summarized in table (2).

Vaccination schedule was studied in relation to anti-HBs titer as shown in table (2). Positive high protective titer was significantly related to vaccine schedule completion (p value <0.001) and compulsory vaccination with booster doses (p value <0.001) and. Low protective titer was statistically significantly related to noncompulsory vaccinated residents (60.8%) with p value <0.001.

Strong relation between the number of vaccination doses and the HBsAb titer was noticed. Negative HBsAb titer was significantly more in residents who received one or two doses of HBV vaccines while positive high protective titer was more in those receiving 5 to 6 doses of vaccines with p value <0.001 and none of the residents who received 5 or 6 doses of the vaccine showed negative antibody titer. Also, a positive correlation was detected between the number of vaccination doses and the high level of HBsAb titer (r = 0.653 and p value <0.001) as well as the number of booster vaccine doses (r = 0.773 and p value <0.001) as presented in table (2) and (3).

Duration of last vaccine received for ≥10 years was statistically significant related to the negative HBsAb protective titre (p value <0.001) also with negative correlation between the level of protective titer and the duration of last vaccine received (r = -0.424 and p value 0.002) as presented in table (2) and (3).

**Regression analysis in relation to HBsAb protective titer**

All the statistically significant values (gender, smoking, vaccination status, duration since last vaccine dose) were added for multivariate logistic regression to detect factors that independently affect the HBsAb titer and protection status as shown in table (4). The duration of last vaccination dose (<10 years) and the complete vaccination schedule (≥3 doses) including those who received booster doses are the significant independent factors for HBsAb positivity with 99.2% & 86% protection with p value <0.001 and 0.012 respectively and those who had vaccine duration ≥10 years and incomplete vaccination (2 or less doses) were 125 and 6 times more likely for being un protective respectively.
### Table 1. HBV vaccination doses in the studied population

<table>
<thead>
<tr>
<th>Doses</th>
<th>Compulsory vaccines only (N=17)</th>
<th>Compulsory vaccines + Booster (N=116)</th>
<th>Non compulsory vaccines (N=69)</th>
<th>All the studied population (N=202)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>29.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>70.6</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>14.6</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>65.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Baseline characteristics and vaccination status of the studied population in relation to HBsAb titer

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative HBsAb titer (&lt;10 IU/L)</th>
<th>Positive low HBsAb titer (10-100IU/L)</th>
<th>Positive high HBsAb titer (&gt;100IU/L)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Male (N = 79)</td>
<td>15</td>
<td>15.5%</td>
<td>40</td>
<td>41.20%</td>
</tr>
<tr>
<td>Female (N = 105)</td>
<td>4</td>
<td>3.8%</td>
<td>31</td>
<td>29.50%</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td>0.354</td>
</tr>
<tr>
<td>Rural (N = 25)</td>
<td>2</td>
<td>8.0%</td>
<td>12</td>
<td>48.00%</td>
</tr>
<tr>
<td>Urban (N = 177)</td>
<td>17</td>
<td>9.6%</td>
<td>59</td>
<td>33.30%</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No (N = 168)</td>
<td>10</td>
<td>6.00%</td>
<td>58</td>
<td>34.50%</td>
</tr>
<tr>
<td>Yes (N = 34)</td>
<td>9</td>
<td>26.50%</td>
<td>13</td>
<td>38.20%</td>
</tr>
<tr>
<td>Needle stick injury</td>
<td></td>
<td></td>
<td></td>
<td>0.094</td>
</tr>
<tr>
<td>No (N = 97)</td>
<td>0</td>
<td>0%</td>
<td>15</td>
<td>42.9%</td>
</tr>
<tr>
<td>Yes (N = 105)</td>
<td>19</td>
<td>11.4%</td>
<td>56</td>
<td>33.5%</td>
</tr>
<tr>
<td>Non Compulsory vaccination (N = 69)</td>
<td>7</td>
<td>10.2%</td>
<td>42</td>
<td>60.8%</td>
</tr>
<tr>
<td>Compulsory vaccination (N =133)</td>
<td>12</td>
<td>9%</td>
<td>29</td>
<td>21.8%</td>
</tr>
<tr>
<td>Complete vaccination</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No (N = 45)</td>
<td>12</td>
<td>26.6%</td>
<td>32</td>
<td>71.2%</td>
</tr>
<tr>
<td>Yes (N = 157)</td>
<td>7</td>
<td>4.5%</td>
<td>39</td>
<td>24.8%</td>
</tr>
<tr>
<td>Booster dose after compulsory</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No (N = 17)</td>
<td>9</td>
<td>53%</td>
<td>7</td>
<td>41.2%</td>
</tr>
<tr>
<td>Yes (N = 116)</td>
<td>3</td>
<td>2.6%</td>
<td>22</td>
<td>19%</td>
</tr>
<tr>
<td>Last vaccination date</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ 10 years (N = 28)</td>
<td>17</td>
<td>60.7%</td>
<td>10</td>
<td>35.7%</td>
</tr>
<tr>
<td>&lt; 10 years (N= 174)</td>
<td>2</td>
<td>1.2%</td>
<td>61</td>
<td>35%</td>
</tr>
</tbody>
</table>
Table 3. Correlation between total number of vaccine doses, number of booster vaccines doses, vaccination duration and HBsAb titer in the studied population

<table>
<thead>
<tr>
<th>HBsAb titer</th>
<th>R</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of vaccine doses</td>
<td>0.653</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of booster vaccine doses</td>
<td>0.773</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Last vaccination date</td>
<td>-0.424</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 4. Multivariate Logistic regression analysis for HBsAb titer in the studied population

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>P value</th>
<th>OR</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>4.83</td>
<td>0.858</td>
<td>&lt;0.001</td>
<td>125.2</td>
<td>23.3</td>
<td>672.5</td>
</tr>
<tr>
<td>Vaccine completion</td>
<td>1.9</td>
<td>0.77</td>
<td>0.012</td>
<td>6.94</td>
<td>1.5</td>
<td>31.4</td>
</tr>
</tbody>
</table>

B=Regression coefficients, SE=Standard error of the coefficient, OR=Odds Ratio. 95% CI for OR = 95% confidence interval for the Odds Ratio. P-value≤0.05 is considered significant

Figure 1. HBV vaccination schedule of the studied population

Total population (n = 202)

- Compulsory vaccination
  - 65.8% (n= 133)

- Non compulsory vaccination
  - 34.2% (n= 69)

- Booster vaccination
  - 87.2% (n = 116)

- No booster vaccination
  - 12.8% (n= 17)
Discussion

The presence of strong immunological memory cells plays an important role in long term protection against HBV [9]. The higher concentrations of post vaccination serum antibody might lead to longer duration of immunity, but the exact duration remains unknown [10, 11]. Thus, the assessment of anti-HBs status after immunization is very important especially among high-risk groups as health care workers [12].

Vaccine completion among our studied residents reached 77.7%. Our results were higher than the study conducted in Microbiology laboratory workers in Pakistan who found that only 59.4% of the residents and junior doctors had received complete vaccination [13]. This reflects the awareness about HBV vaccination in Egypt through compulsory vaccination or encouraging noncompulsory vaccination programs. In Egypt, the HBV vaccination program was applied in 1992 with a schedule of 2, 4 and 6 months of age. Several studies indicated that this program was effective in production and maintenance of long term protection against hepatitis B reaching up to 16 years in one study [14,15].

An interesting observation in our results was that 82.7% of the residents were exposed to needle stick injury irrespective of their medical specialty. Occupational exposure to percutaneous injuries and Needle stick injuries are a substantial source of infections with blood-borne pathogens among health care workers [16]. These results highlighted the importance of safety measures and infection control in health care workers and the need of HBV vaccination and assessment of protective titer.

Only 9.4% of our studied population had negative protective titre (<10 IU/l) against HBV infection. Makvandi et al. (2015) who studied HBsAb and Specific Gamma Interferon response in health care workers after vaccination in Iran found that 5% to 7% of the population are nonresponsive to vaccine [17]. Closely similar results were observed by [9] with overall percentage 5.2% and [18] in Iran with 12.8%.

Among our studied population, negative HBsAb titer was significantly more in male residents (15.5%) and smokers (26.5%). Similar to our results Platkov and colleagues in 2003 has documented that hepatitis B vaccine has decreased its immunogenicity with increasing age, obesity, smoking and male gender [9]. Lakshmanan et al. (2017) concluded that 24% of the studied smokers showed antibody titer below 10 IU/L [18].

The relation between the gender and the level of protective antibodies remains unclear, many studies attributed that to the estrogen and progesterone hormones with no confirmatory data available [19,20]. Cigarette smoking is associated with wide range of alterations in immune function. It has been supposed that diminished response in smokers maybe due to the increasing of T suppressor lymphocytes [21].

In the current study HBsAb titer was significantly more in completely vaccinated group than incompletely vaccinated and the number of vaccine doses was positively correlated with positive high protective titer. This result goes in agreement with Jha et al. (2012) and El-Melligy et al. (2016) who found that the HBV vaccine protection is less in those who received 1 or 2 dose vaccine compared to those who received full vaccination doses (3doses) [12,22].

Residents who received booster doses of vaccines after compulsory vaccination achieved high positive HBsAb titer than compulsory vaccination only. Similar results were observed by Platkov et al. (2003) and Shooshtari et al. (2015) [9,10]. Lower HBsAb titer in only compulsory vaccinated residents may be due to duration elapsed since last time of vaccination which raised the problem of immunity waning over time.

This raise the importance of universal booster vaccination for high-risk groups like health care workers who were non responders (immunity < 10IU/l) or hyporesponders (immunity < 100 IU/l) [17]. Many research don’t recommend giving booster dose as long as the subject is immunocompetent and achieved positive titer. However, many others recommend a booster dose following compulsory immunization in high-risk group and immunocompromised individuals (Lakshmanan et al., 2017) [18].

In our study there was a positive correlation between the number of booster doses and the positive high HBsAb titer, however the exact number of booster doses required for reaching high antibody protection following full compulsory vaccine could not be determined statistically. Previous study recommended at least 2 doses of booster vaccines for at-risk vaccinated adults
without persistent protection or with low protective titers [19].

We concluded that vaccine duration was strongly related to negative and low protective titers. Similar to our results an Iranian study conducted on health care workers who received HBV vaccination more than 18 years showed a declining trend in anti-HBsAb titers over the time after vaccination [23]. Also an Egyptian study among health care workers revealed that 33% of the studied groups were non responders of HBV vaccine after 5-10 years of post vaccination period [10].

The declining trend of anti-HBsAb levels with duration of vaccination which was reported in this study shows the importance of measuring the HBsAb titer in health care workers following full vaccination because of the various responses to hepatitis B vaccine among different individuals, ranging from non responders to weak or highly responsive.

In conclusion, since the persistence of protective anti-HBsAb level is related to the number of years elapsed since vaccination and the number of vaccine doses, so assessment of the vaccination status by measuring HBsAb after immunization among health care workers is important for proper identification of their level of protection against hepatitis B infection.

Limitations of the study
This study was conducted in a single center with a small sample size. Additionally, duration between last vaccination dose given and the time of the study was largely variable affecting the accuracy of data generated.

Conflict of interest
The authors report no conflicts of interest in this work.

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