Humoral Immune Memory to Hepatitis B Vaccine after Primary Vaccination of Children and Adolescents in Assiut, Egypt

Iman I. Salama¹ *, Samia M. Sami², Safaa M. Elseroug³, Hanaa M. Eman⁴, Somaia I. Salama¹, Hazem M. Elhariri¹, Samia A. Hemeda¹, Amal I. Hassanain⁵, Aida M. Abdel Mohsen¹, Walaa A. Fouad¹, Lobna A. El Etreby¹ and Zeinab N. Said⁵

¹Community Medicine Research Department, National Research Center, Cairo, Egypt
²Child Health Department, National Research Center, Cairo, Egypt
³Department of Environmental and Occupational Medicine, National Research Center, Cairo, Egypt
⁴Dermatology and Venereology, National Research Center, Cairo, Egypt
⁵Microbiology and Immunology Department, Faculty of Medicine (for Girls), Al-Azhar University, Cairo, Egypt

ABSTRACT

Objectives: We sought to assess the prevalence of hepatitis B virus (HBV) seroprotection among vaccinated children in the Assiut governorate, Egypt, and assess a booster dose immune memory response among non-seroprotected children.

Methods: Using a multistage cluster sample, 566 children were recruited from three clusters: one urban and two rural. Children were aged from nine months to 16 years old. All participants received the full three doses of the compulsory HBV vaccine during infancy. Serum hepatitis B surface antigen (HBsAg), total anti-hepatitis B core (anti-HBc) antibodies, and quantitative detection of anti-HBs were measured using enzyme-linked immunosorbent assay. Repeatedly positive samples for HBsAg/anti-HBc were submitted for quantitative HBV DNA detection using real-time polymerase chain reaction. Non-seroprotective participants (anti-HBs < 10 IU/L) were given a booster dose of HBV vaccine. Two weeks later, a blood sample was taken from each child to assess an anamnestic response.

Results: The seroprotection rate was 53.2%, and only two children had HBV breakthrough infection (0.4%) with positive serum anti-HBc and HBV DNA. Age was the only significant predictor for non-seroprotection with an adjusted odds ratio (OR) of 3.2, 9.4, and 9.9 among children aged 5–10, 11–15, and > 15 years, respectively, compared to younger children (p < 0.001). About 85% of non-seroprotected children developed an anamnestic response after receiving the booster dose, and 84.3% of responders had a good response (≥ 100 IU/L). Undetectable pre-booster titer was found to be the only risk factor for non-response to booster with OR = 3.2 (p < 0.010). About 95.7% of children who were not responding to booster dose developed immune response after receiving the three doses of HBV vaccine.

Conclusions: Older age of children was the only significant predictor for HBV non-seroprotection. High anamnestic response rate signifies the presence of immune memory with long-term protection despite the waning of anti-HBs over time. However, some children with pre-booster undetectable anti-HBs titers may be unable to develop anamnestic response, and a second vaccination series might be necessary for HBV protection for these children.

Keywords: Hepatitis B Surface Antigens; Hepatitis B virus; Egypt; Hepatitis B Antibodies; Immunization, Secondary.

ORIGINAL ARTICLE

Oman Medical Journal [2020], Vol. 35, No. 5: e175

Chronic hepatitis B virus (HBV) infection is a major global public health problem and a serious risk factor for deaths due to the development of cirrhosis or hepatocellular carcinoma.¹ In Egypt, there are between two to three million chronic HBV carriers.² Vaccination is the most effective measure to decrease the incidence of both HBV infection and hepatocellular carcinoma. Compared to other measures, vaccination is more cost-effective and cost-beneficial.³

Egypt adopted the global strategy for control of HBV by implementing routine infant HBV vaccination since October 1992.⁴ The 2015 Egyptian Health Issues Survey reported a high coverage rate of HBV vaccination, as 95% of children had received the full three doses of HBV vaccine.⁵ HBV vaccines are immunogenic in newborns and infants and provide
high seroprotection rates in early childhood. Almost all vaccinated infants (92.6–100%) have mounted seroprotective anti-hepatitis B surface (anti-HBs) antibody concentrations (≥10 IU/L) one to three months after the three-dose schedule.6,7

Immune memory is virtually present in all vaccines when a challenge dose of vaccine is given four to six years post-vaccination. However, 10–30% of those vaccinated during infancy do not respond to a booster dose of the vaccine by 14–20 years old. This failure to respond reduces the likelihood of having short or long-term anamnestic responses, which may offer a window of chance for transmission of HBV.8–10

Estimating the incidence of breakthrough infection (positive anti-hepatitis B core (anti-HBc) antibody/ HBV DNA) as well as chronic carrier state (positive HBs antigen (HBsAg)) among previously vaccinated individuals is a practical method to determine the long-term protection of the HBV vaccine.11 However, Egypt lacks assessment of long-term seroprotection among older children and adolescents as well as early seroprotection one to three months after compulsory vaccination.

Therefore, there was an imperative need to assess the national HBV vaccination program in Egypt. We conducted a community-based study to provide a real guide to decision-makers, particularly the Ministry of Health and population (MOHP). It is valuable to assess the situation in southern Egypt, which has some structural and financial barriers to health services. This study aimed to determine the short- and long-term effectiveness of HBV vaccine and to assess the anamnestic response rate to a booster dose of the vaccine in non-seroprotected children.

**METHODS**

The Medical Research Ethics Committee of National Research Center and the Ethical Committee of Ministry of Health approved the study protocol. Approval was also obtained from the Ministry of Education. Signed written informed consent was obtained from all parents a few days before the interview. Adolescents > 10 years were also interviewed after their verbal consent.

The present work is a part of a national community-based epidemiologic research project in six governorates representing all geographic regions of Egypt from July 2010 to June 2013. This study represents its part in the Assiut governorate, which is located in the Nile valley in Upper Egypt, 375 km south of the capital city, Cairo.

We recruited children using a multistage cluster sampling technique. Probability proportional to size sampling and consideration of the population size of each governorate was utilized in sampling and cluster selection. In the Assiut governorate, three clusters were randomly chosen from an urban place (Assiut city) and two rural places (El-Dwina village in Abu Tij and kom-Abosheel village in Abnub). Cooperative efforts with authorities of MOHP at the facility level were provided to manage the work and make arrangements. Five facilities were randomly selected in each cluster: maternal and child health center (MCHC)/health units, kindergarten, primary, preparatory and secondary schools.

Data and blood samples were collected from the participants in the first visit. Serum was separated from each sample to analyze anti-HBV antibody levels. Participants with non-protective levels of anti-HBV antibodies received a booster dose of HBV monovalent Euvax vaccine (Korean origin) in the second visit. In the third visit, a second blood sample was taken from recipients of the booster dose and re-assessed for anti-HBV antibody levels.

A total of 566 children were enrolled in the study: 247 boys and 319 girls, aged from nine months to 16 years. All participants had received the three compulsory doses of HBV vaccine during infancy. There were no exclusion criteria.

A closed-end questionnaire was designed and submitted for pilot testing. For quality assurance in each cluster, training sessions for supervisors, interviewers, and MOHP staff were held. Peel-off barcode sheets were used. Face-to-face interview was carried out with parents or caretakers for children aged < 12 years, but directly with preparatory or secondary school children. Demographic data included age, sex, and socioeconomic status (SES). Data were also collected concerning child HBV vaccination status, and the available vaccination card was revised for date and dose intervals of the HBV vaccine. SES was determined according to Fahmy and Sherbiny.12 It depends on parents’ education, parents’ working status, water source, sewage disposable, electricity, and some modifications of family income. Height and weight were measured to assess the nutritional status of the participants.

Blood samples (3–5 mL) were withdrawn aseptically from all participants. Serum was
separated from each sample, divided into two labeled aliquots, and stored at −20 °C. Detection of HBV markers was carried out in the Virology Laboratory of the Microbiology and Immunology Department, Faculty of Medicine (for girls), Al-Azhar University, Cairo. Qualitative determination of serum total anti-HBc antibody, HBsAg, and quantitative detection of serum anti-HBs were assessed using commercially available enzyme-linked immunoassays (ELISA, DiaSorin, Italy) according to the manufacturer instructions. For quality assurance, re-assessment of cases found to be positive for anti-HBc or HBsAg was done. According to international standards, anti-HBs ≥ 10 IU/L was considered to be protective against HBV infection.\textsuperscript{13}

Repeatedly, positive samples for either anti-HBc or HBsAg were submitted for quantitative detection of HBV DNA by real-time polymerase chain reaction (PCR) using automated system. Viral DNA was extracted from serum samples using QIAxtractor\textsuperscript{®}, and VX kit as recommended by the manufacturer (QIAGEN, Germany). PCR setup was automated via QIAgility (QIAGEN, Germany). HBV real-time assays were performed in combination with Artus HBV RG PCR Kit (Artus\textsuperscript{®}GmbH, Hamburg, Germany) and the real-time PCR instrument, Rotor-Gene Q (QIAGEN, Germany). The thermal profile was set according to the manufacturer guidelines. The detection limit of the HBV DNA assay was 3.8 IU/L as per the World Health Organization (WHO) international standard (97/750).\textsuperscript{14} At least two negative controls, one non-template control, and four standards (provided by the manufacturer) were added per run. Strict precautions were considered to control possible contamination. Only reproducible data that revealed no false-positive results in the negative controls were used.

Children with non-protective levels of anti-HBs titers received a booster dose of HBV vaccine as 10 μg of monovalent Euvax HB vaccine, intramuscularly in the deltoid muscle. Two weeks later, a blood sample was taken to measure the post-booster anti-HBs level and assess early anamnestic response. Anamnestic response is defined as a rise in anti-HBs to ≥ 10 U/L.\textsuperscript{13} All children showing antibody response < 10 IU/L (n = 27) were then offered an additional complete course of vaccination (second vaccination series), and 23 of children assessed one month after receiving the three doses.

Data entry was carried out using an Excel sheet, and statistical analysis using SPSS Statistics (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.). The geometric mean titer (GMT) was calculated to indicate the central tendency of anti-HBs titers in consideration of the skewed distribution of anti-HBs level. Children who had an undetectable anti-HBs titer were assigned a nominal serum anti-HBs-titer value of 0.05 IU/L to calculate GMT.\textsuperscript{10} Chi-square was computed for comparisons of percentages. The odds ratio (OR) was calculated for 2 × 2 frequency tables (binary variables). In the case of categorical variables (> 2 groups e.g., age groups), OR was calculated for each group compared to a reference group (e.g., OR was calculated for each of the 5-10, 11-15, > 15 years groups and compared to the < 5 years group). The McNemar test was done for two related percentages (pre- and post-booster).

Multivariate logistic analysis was performed to explore significant predictors of non-seroprotected and post-booster non-response. The alpha level of significance was set at $p < 0.050$.

RESULTS

A total of 566 children with comparable age group and gender ($p > 0.050$) were included in this study in the three studied clusters [Table 1]. Breakthrough infection (positive anti-HBc and HBV DNA) was detected in only two children (2/566, 0.4%). Both had anti-HBs that exceeded 100 IU/L, and none of them were positive for HBsAg (occult infection). They were a 10-year-old boy and an 11-year-old girl from Assiut city. Both remained positive for anti-HBc and negative for HBsAg after one-year follow-up (data not shown).

The non-seroprotection rate (anti-HBs < 10 IU/L) was 46.8% (264/564). The participation rate of non-seroprotected children who came to receive the booster dose was 75.4% (199/264), and the donation rate for a post-booster blood sample was 89.4% (178/199). Females showed a higher non-seroprotection rate than males (51.6% vs. 40.7%, respectively). Increasing age represented a high risk for non-seroprotection as the OR was 3.0, 9.5, and 9.6-times in the older age groups (5–10, 11–15, and >15 years, respectively) compared to the youngest
group (< 5 years). Noticeably, the older age groups (10–15, ≥ 15 years) were similar with regards to the non-seroprotection rate and adjusted OR (aOR). All other studied variables seemed not to have any significant role with regards to the non-seroprotection rate [Table 2].

Table 1: Characteristics of the participants in three clusters of Assiut governorate.

| Variables                      | Total       | Assiut city (Urban) | Abu Tij village (Rural) | Abnub village (Rural) | p-value |
|--------------------------------|-------------|----------------------|-------------------------|-----------------------|---------|
| Sex                            |             |                      |                         |                       |         |
| Boys                           | 246 (43.6)  | 74 (39.8)            | 78 (41.5)               | 94 (49.5)             | 0.128   |
| Girls                          | 318 (56.4)  | 112 (60.2)           | 110 (58.5)              | 96 (50.5)             |         |
| Age, years                     |             |                      |                         |                       |         |
| < 5                            | 191 (33.9)  | 65 (34.9)            | 67 (35.6)               | 59 (31.1)             | 0.920   |
| 5–10                           | 96 (17.0)   | 32 (17.2)            | 28 (14.9)               | 36 (18.9)             |         |
| 11–15                          | 168 (29.8)  | 52 (28.0)            | 58 (30.9)               | 58 (30.5)             |         |
| > 15                           | 109 (19.3)  | 37 (19.9)            | 35 (18.6)               | 37 (19.5)             |         |

Data presented as n (%). p significant at > 0.050.

Table 2: Anti-hepatitis B surface (anti-HBs) antibody level as regards some demographic variables among studied children.

| Variables                      | Total      | anti-HBs < 10 IU/L | anti-HBs ≥ 10 IU/L | p-value | OR (95% CI) |
|--------------------------------|------------|--------------------|--------------------|---------|-------------|
| Sex                            |            |                    |                    |         |             |
| Boys                           | 246        | 100 (40.7)         | 146 (59.3)         | 0.010*  | -           |
| Girls                          | 318        | 164 (51.6)         | 154 (48.4)         | 1.6 (1.1–2.2) |             |
| Age, years                     |            |                    |                    |         |             |
| < 5                            | 176        | 31 (17.6)          | 145 (82.4)         | 0.001** |             |
| 5–10                           | 97         | 38 (39.2)          | 59 (60.8)          | 3.0 (1.7–5.3) |             |
| 11–15                          | 159        | 106 (66.7)         | 53 (33.3)          | 9.5 (6.0–15.6) |             |
| > 15                           | 132        | 89 (67.4)          | 43 (32.6)          | 9.6 (5.7–16.4) |             |
| Weight for age Z score         |            |                    |                    |         |             |
| < -2 SD                        | 49         | 21 (42.9)          | 28 (57.1)          | 0.625   | 0.8 (0.5–1.5) |
| ≥ -2 SD                        | 515        | 243 (47.2)         | 272 (52.8)         |         |             |
| Height for age Z score         |            |                    |                    |         |             |
| < -2 SD                        | 108        | 53 (49.1)          | 55 (50.9)          | 0.794   | 1.0 (0.6–1.5) |
| ≥ -2 SD                        | 398        | 200 (50.3)         | 198 (49.7)         |         |             |
| SES                            |            |                    |                    |         |             |
| Very low                       | 219        | 112 (51.1)         | 107 (48.9)         | 0.061   | 1.4 (0.9–2.2) |
| Low                            | 100        | 52 (52.0)          | 48 (48.0)          | 1.4 (0.8–2.4) |             |
| Middle                         | 129        | 49 (38.0)          | 80 (62.0)          | 0.8 (0.4–1.3) |             |
| High                           | 108        | 47 (43.5)          | 61 (56.5)          |         |             |
| Residence                      |            |                    |                    |         |             |
| Urban                          | 186        | 93 (50.0)          | 93 (50.0)          | 0.333   | -           |
| Rural                          | 378        | 171 (45.2)         | 207 (54.8)         | 1.2 (0.9–1.7) |             |
| Cluster                        |            |                    |                    |         |             |
| Assiut                         | 186        | 93 (50.0)          | 93 (50.0)          | 0.317   | 1.3 (0.9–2.0) |
| Abu Tij                        | 188        | 90 (47.9)          | 98 (52.1)          | 1.2 (0.8–1.9) |             |
| Abnub                          | 190        | 81 (42.6)          | 109 (57.4)         |         |             |

Data presented as n (%). *p < 0.050, **p < 0.010; OR: odds ratio; CI: confidence interval; SD: standard deviation; SES: socioeconomic status.
In the logistic regression analysis, age, gender, SES, urban/rural, and cluster were entered as independent variables and anti-HBs non-seroprotection rate as the dependent variable. Age was the only significant predictor in the regression model. The older age groups (5–10, 11–15, and >15 years) exhibited a significant high aOR of non-seroprotection compared to children aged < 5 (3.2, 9.4, and 9.9, respectively). The gender variable was included in the model despite being non-significant while the other non-significant variables were excluded [Table 3].

About 84.3% of the non-seroprotected children (150/178) developed anamnestic response to booster dose, and only 15.7% were non-responders. Post-

### Table 3: Predictors for risk of non-seroprotective level using logistic regression.

| Variables                  | B    | SE   | Wald  | p-value | aOR (95% CI) |
|----------------------------|------|------|-------|---------|--------------|
| Female sex                 | 0.263| 0.197| 1.789 | 0.181   | 1.3 (0.9–1.9) |
| Age groups, years          |      |      |       |         |              |
| < 5                        |      |      | 96.106| 0.001** |              |
| 5–10                       | 1.154| 0.292| 15.642| 0.001** | 3.2 (1.8–5.6) |
| 11–15                      | 2.243| 0.262| 73.148| 0.001** | 9.4 (5.6–15.7) |
| > 15                       | 2.296| 0.273| 70.508| 0.001** | 9.9 (5.8–17.0) |
| Constant                   | -1.569| 0.201| 61.090| 0.001** | 0.2 (-)     |

SE: standard error; **p < 0.010; aOR: adjusted odds ratio; CI: confidence interval.

Data presented as n (%).

### Table 4: Early anamnestic response among children with pre-booster non-seroprotective levels as regards studied demographic variables.

| Variables                        | Total N = 178 | No-response anti-HBs < 10 IU/L n = 28 | Anamnestic response anti-HBs ≥ 10 IU/L n = 150 | p-value | OR (95% CI) |
|----------------------------------|---------------|---------------------------------------|-----------------------------------------------|---------|-------------|
| Pre-booster anti-HBs (IU/L)      |               |                                       |                                               |         |             |
| Undetected (<1)                  | 66            | 17 (25.8)                             | 49 (74.2)                                     | 0.005** | 3.2 (1.4–7.3) |
| Detected (≥1)                    | 112           | 11 (9.8)                              | 101 (90.2)                                    |         |             |
| Sex                              |               |                                       |                                               |         |             |
| Boys                             | 66            | 8 (12.1)                              | 58 (87.9)                                     | 0.310   | 0.6 (0.3–1.5) |
| Girls                            | 112           | 20 (17.9)                             | 92 (82.1)                                     |         |             |
| Age, years                       |               |                                       |                                               |         |             |
| < 5                              | 16            | 0 (0.0)                               | 16 (100)                                      | 0.102   | -           |
| 5–10                             | 21            | 1 (4.8)                               | 20 (95.2)                                     |         |             |
| 11–15                            | 76            | 14 (18.4)                             | 62 (81.6)                                     |         |             |
| >15                              | 65            | 13 (20.0)                             | 52 (80.0)                                     |         |             |
| SES                              |               |                                       |                                               |         |             |
| Very low                         | 75            | 12 (16.0)                             | 63 (84.0)                                     | 0.890   | -           |
| Low                              | 36            | 7 (19.4)                              | 29 (80.6)                                     |         |             |
| Middle                           | 32            | 4 (12.5)                              | 28 (87.5)                                     |         |             |
| High                             | 33            | 5 (15.2)                              | 28 (84.8)                                     |         |             |
| Residence                        |               |                                       |                                               |         |             |
| Urban                            | 58            | 9 (15.5)                              | 49 (84.5)                                     | 0.957   | 1.0 (0.4–2.3) |
| Rural                            | 120           | 19 (15.8)                             | 101 (84.2)                                    |         |             |
| Cluster                          |               |                                       |                                               |         |             |
| Assiut city                      | 58            | 9 (15.5)                              | 49 (84.5)                                     | 0.574   | -           |
| Abu Tij                          | 69            | 13 (18.8)                             | 56 (81.2)                                     |         |             |
| Abnub                            | 51            | 6 (11.8)                              | 45 (88.2)                                     |         |             |

Data presented as n (%).

** p < 0.010; anti-HBs: anti-hepatitis B surface antibody; OR: odds ratio; CI: confidence interval.

* number of the studied children was 176 as socioeconomic status data were missing for two children.
booster anti-HBs showed highly significant higher levels (anamnestic response) compared to their pre-booster mean level (123.3±20.1 vs. 0.6±7.6 IU/L, respectively, \( p < 0.001 \)). Post-booster anti-HBs levels showed a highly significant positive correlation with pre-booster levels (\( r = 0.303, p < 0.001 \)).

Table 4 shows early anamnestic response among children with pre-booster non-seroprotective levels with regards to the studied demographic variables. Pre-booster undetectable level of anti-HBs was the only significant risk factor for being non-responding to challenging booster dose, \( p < 0.010 \). Moreover, using logistic regression analysis, pre-booster undetectable level of anti-HBs was the only significant predictor for being non-responding to booster dose (OR = 3.2, 95% confidence interval (CI): 1.4–7.3). Among non-responders to the booster dose, 22/23 children (95.7%) developed an immune response after receiving the three doses of the second vaccination series. One child, aged 16, had immunological second vaccination failure and post-booster undetectable anti-HBs.

There was a significant difference in the percentages of anti-HBs groups in the three clusters at Assiut governorate as regards pre- and post-booster dose. Regarding anti-HBs pre-booster, a high percentage of non-seroprotected children was noted in the three cities, especially Assiut and Abu Tij, and to a lesser extent in Abnub (50.0%, 47.9%, and 42.6%, respectively). Post-booster, a significantly higher percentage of the non-seroprotected children who received the booster dose were rendered highly seroprotected (81.0%, 60.9%, 62.7%, respectively) (data not shown).

**DISCUSSION**

The success of national immunization programs for routine childhood vaccination against hepatitis B depends on the continuity of protection during adolescence and adulthood when chances of exposure to HBV increase, whether through the sexual route or due to other risk behaviors.\(^{15}\) Various long-term follow-up studies have shown that immune memory persists in healthy individuals vaccinated during infancy with HB monovalent or HB combination vaccines used in various primary series regimens even after circulating neutralizing anti-HBs disappear.\(^{16-19}\)

This study revealed that the non-seroprotection rate was 46.8%. Female gender and increasing age represented a significant high risk for non-seroprotection. This might indicate the progressive waning of vaccine-conferred immunity against HBV infection with the increased duration since receiving the primary vaccination. However, even if anti-HBs concentrations decline to < 10 IU/L, the immune memory continues to persist. Persistence of this vaccine-induced immune memory has been demonstrated among 84.3% of non-seroprotected children who developed anamnestic response after receiving the booster dose. Nearly all the non-responder to booster dose (26/27) children were aged ≥ 10 years old, and they might be at risk of getting HBV infection. Afifi et al,\(^{20}\) found that among children aged 6–11 years, 39.3% had a protective level of anti-HBs. The mean level of anti-HBs decreased significantly with increasing age (\( p = 0.026 \)). A significant negative correlation was found between current age and anti-HBs levels (\( p < 0.005 \)). No significant difference was detected between males and females or among different levels of SES (\( p > 0.050 \)). Non-seroprotection reached a maximum at the 10–15, >15 age groups compared to younger ages.\(^{21}\)

Recent evidence indicating that immune memory may start to fade in vaccinated individuals after the second decade arouses interest to discover whether a booster dose is needed to sustain long-term immunity or not.\(^{22,23}\) Post-booster anti-HBs levels indicated a highly significant positive correlation with pre-booster levels (\( r = 0.303, p < 0.001 \)). The marked rise in titer of non-seroprotection after a booster dose suggested that children have a competent anamnestic response. In agreement with our findings, Saffar and Rezai,\(^{24}\) found that nearly 42% of HB vaccinated children at birth were not seroprotected at the age of 10–11 years, but most boosted subjects (87.3%) reserved robust immunologic memory and quickly regained a protective anti-HBs antibody titer. Jafarzadeh and Montazerifar,\(^{22}\) also reported that the seroprotection raised to 95.75% in the studied children after booster vaccination. The non-seroprotected rate for anti-HBs rose from about 1% to 63% at the ages of one and 15–17 years, respectively.

Salama et al,\(^{25}\) reported that among children having undetectable anti-HBs titers, a HBV booster dose vaccine might be unable to induce sufficient immunological response. In our study, 22/23 children (95.7%) who were non-responders to the booster dose developed an immune response after
receiving the three doses of the second vaccination series. Only one child had immunological second vaccination failure and post-booster undetectable anti-HBs. Similar results were reported by Lu et al. They reported that three-dose revaccination was effective, and the developed immune response was correlated with the pre-booster titer and level > 1.0 IU/L to ensure an effective immune response.

Persistent memory exceeding years can be detected by a rapid increase in anti-HBs level after booster vaccination, even in those with untraceable anti-HBs. Previous studies performed up to five years after vaccination showed that loss of detectable antibodies does not essentially indicate loss of protection. When boosted, most participants in those studies who had an anti-HBs concentration of < 1 IU/L showed a strong, fast anamnestic response, signifying the presence of a specific immune memory. However, in the present study, using logistic regression analysis, it was found that pre-booster undetectable levels of anti-HBs was the only significant predictor for being non-responding to challenging booster dose (OR = 3.2, 95% CI: 1.4–7.3). Other variables such as age, gender, SES, and residence were not predictors for non-response. The association between pre- and post-booster anti-HBs levels seems to follow a horse race effect as the higher anti-HBs level pre-booster developed higher post-booster anti-HBs.

The 2015 Egypt Health Issues Survey revealed that the percentages of positive anti-HBc and HBsAg were 0.7% and 0.1% among children aged 1–14 years while they were 8.0% and 1.0% among the population aged 1–59 years, respectively. They stated that: “the rapid expansion of the coverage of hepatitis B vaccinations following their addition to the national immunization program in the 1990s likely means that the low hepatitis B infection rates observed among children and young adults will be the norm among older cohorts as well in the future”. We found breakthrough infection among 0.4% of the studied children. Children who had positive anti-HBc and HBV DNA with the absence of HBsAg up to one-year follow-up indicates occult HBV infection despite incorporating HBV vaccination in infant immunization schedules in Egypt since 1992. Similarly, several studies in countries with very high HBV prevalence revealed that a proportion of fully vaccinated infants had benign breakthrough HBV infections, defined as anti-HBc seropositivity in vaccinated participants who were not chronically infected. Zanetti et al. found that isolated anti-HBc without the development of HBsAg carrier state occurred in 1.3% of children. Since these children were born to HBsAg-negative mothers, it is possible that they became infected after primary vaccination and that vaccination partially helped to protect these children, so they suffered only from subclinical transient infections when exposed to HBV. If there is no clinical disease, the presence of anti-HBc alone may be due to false-positive test results or occult infection with the presence of HBV DNA less than the detectable limit of the currently available assays. Sadeghi et al. observed that some immunized children cleared serum HBV DNA, accompanied by anti-HBc disappearance with no elevated serum levels of anti-HBs in 36 months. Wang et al. found that many HBsAg-negative with positive anti-HBc children, especially among those born to HBsAg-negative mothers, turned negative anti-HBc within 12–18 years, suggesting that the HBV infection was most likely cleared.

The crucial strength point of the current work is that it is a community-based study that measures and evaluates the long-term protection of the HBV vaccination program implemented in Egypt since 1992. It involved a large sample size of children vaccinated during infancy from a representing geographic distribution in Assuit governorate with a wide age range (9 months to 16 years). Moreover, the same recombinant HBV vaccine used in primary vaccination was used for the booster dose as well as the second vaccination series. So, it assesses the humoral immunity induced by the primary vaccination and after receiving a booster dose and revaccination for booster dose non-responders, respectively.

Although the vaccination history of the majority of participants was obtained from valid vaccination cards, a few cases depended on the recall of their parents, which could be a source of recall bias. However, there was no difference in the distributions of pre- and post-booster anti-HBs titers among participants with and without vaccination cards, indicating minimal recall bias and hence the validity of the findings. Moreover, vaccination coverage in the studied areas at the time of the study was over 95%. In addition, the lack of the base-line immune response to primary HBV vaccination, participants who did not respond to booster dose might be considered as non-responders to primary vaccination.
or initial responders who lost their immunological memory to HBV vaccine.

**CONCLUSION**

Older age of children was the only significant predictor for HBV non-seroprotection. High anamnestic response rate signifies the presence of immune memory with long-term protection despite the waning of anti-HBs over time. However, some children with pre-booster undetectable anti-HBs titers may be unable to induce immunological response, and second vaccination series might be important for these children to increase HBV protection.

Future directions of research would be directed towards assessing the effect of implementing the birth dose – added in 2017 to the Egyptian national HBV vaccination program - on the seroprotection rate and the prevalence of HBV infection among this vaccinated cohort of the Egyptian children.

**Disclosure**

The authors declared no conflicts of interest. This project is funded by the Science and Technology Development Fund (STDF), Egypt. Grant No. 1611.

**Acknowledgements**

The authors are much obliged to the Ministry of Health mainly Dr. Nasr El-Sayed, Dr. Amr Kandil, Dr. Sahar El Shorbagiy in addition to other facility members in Asuit governorate for their profound cooperation, facilitating, preparing and helping us in implementation of the field work. The authors are also grateful to all participants and their parents for their acceptance to participate in the project.

**REFERENCES**

1. WHO. Hepatitis B Fact sheet. 2015 [cited 2015 July]. Available from: http://www.who.int/mediacentre/ factsheets/fs204/en/.

2. El-Zayadi A. Hepatitis B virus infection: the Egyptian situation. Arab J Gastroenterol 2007;8(3):94-98.

3. Zanetti AR, Van Damme P, Shouval D. The global impact of vaccination against hepatitis B: a historical overview. Vaccine 2008 Nov;26(49):6266-6273.

4. El-Razky MS, El-Hawary MA, Salama KM, El-Hennawy AM, Helmy HM, Fahmy ME, et al. Patterns of hepatitis B infection in Egyptian children in the era of obligatory hepatitis B vaccination. Arab J Gastroenterol 2012 Mar;13(1):1-3.

5. Ministry of Health and Population [Egypt], El-Zanaty and Associates [Egypt], and ICF International. 2015. [cited 2015 July]. Available from: https://dhsprogram.com/pubs/pdf/FR313/FR313.pdf. Egypt Health Issues Survey 2015. Cairo, Egypt and Rockville, Maryland, USA: Ministry of Health and Population and ICF International.

6. Keating GM, Noble S. Recombinant hepatitis B vaccine (Engerix-B): a review of its immunogenicity and protective efficacy against hepatitis B. Drugs 2003;63(10):1021-1051.

7. FitzSimons D, Hendrickx G, Vorsters A, Van Damme P. Hepatitis B vaccination: a completed schedule enough to control HBV lifelong? Milan, Italy, 17-18 November 2011. Vaccine 2013 Jan;31(4):584-590.

8. Dentinger CM, McMahon BJ, Butler JC, Dunaway CE, Zanis CL, Bulkow LR, et al. Persistence of antibody to hepatitis B and protection from disease among Alaska natives immunized at birth. Pediatr Infect Dis J 2005 Sep;24(9):786-792.

9. Hammitt LL, Hennessy TW, Fiore AE, Zanis C, Hummel KB, Dunaway E, et al. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine at birth: a follow-up study at 15 years. Vaccine 2007 Sep;25(39-40):6958-6964.

10. Su FH, Cheng SH, Li CY, Chen JD, Hsiao CY, Chien CC, et al. Hepatitis B seroprevalence and anamnestic response amongst Taiwanese young adults with full vaccination in infancy, 20 years subsequent to national hepatitis B vaccination. Vaccine 2007 Nov;25(47):8085-8090.

11. Poorolajal J, Mahmoodi M, Majzdzadeh R, Nasseri-Moghaddam S, Haghdoot A, Fotouhi A. Long-term protection provided by hepatitis B vaccine and need for booster dose: a meta-analysis. Vaccine 2010 Jan;28(3):623-631.

12. Fahmy SI. Determining simple parameters for social classifications for health research. Bull Health Inst Public Health 1983;13:95-108.

13. Zanetti A, Parlato A, Romano L, Desole MG, Ferrara G, Giurandella F, et al. Challenge with a hepatitis B vaccine in two cohorts of 4-7-year-old children primed with hexavalent vaccines: an open-label, randomised trial in Italy. Vaccine 2012 Aug;30(39):5770-5775.

14. Baylis SA, Heath AB, Chudy M, Pisani G, Klorz A, Kerby S, et al. An international collaborative study to establish the 2nd World Health Organization international standard for hepatitis B virus DNA nucleic acid amplification technology-based assays. Vox Sang 2008 May;94(4):358-362.

15. Zanetti A, Desole MG, Romano L, d’Alessandro A, Conversano M, Ferrera G, et al. Safety and immune response to a challenge dose of hepatitis B vaccine in healthy children primed 10 years earlier with hexavalent vaccines in a 3, 5, 11-month schedule: An open-label, controlled, multicentre trial in Italy. Vaccine 2017 Jul;35(32):4034-4040.

16. Said ZN, Abdelwahab KS. Induced immunity against hepatitis B virus. World J Hepatol 2015 Jun;7(12):1660-1670.

17. Xiao J, Zhang J, Wu C, Xao X, Peng G, Peng Z, et al. Impact of hepatitis B vaccination among children in Guangdong Province, China. Int J Infect Dis 2012 Sep;16(9):e692-e696.

18. Ting-Lu Z, Zhi-Ping X, Hong-Yu L, Chang-Hong G, Liang Y, Qiang D, et al. A community-based sero-epidemiological study of hepatitis B infection in Lianyunang, China, 2010. Western Pac Surveill Response J 2012 Aug;3(3):69-75.

19. Saffar H, Ajami A, Saffar MJ, Shojaei J, Sotudeh-Anvari M, Shams-Esfandab K, et al. Prevalence of hepatitis B virus seromarkers in young adults vaccinated at birth; impact on the epidemiology of hepatitis B infection in Iran. Hepat Mon 2014 May;14(5):e17263.

20. Afifi SS, Mahran MH, Said ZN, Salama II, El Khayat H. Serum level of anti-hepatitis B surface antigen among newborns and fully vaccinated infants and children aged 6 to 11 years. Aust J Basic Appl Sci 2009;3(4):3239-3245.

21. Salama II, Sami SM, Said ZN, El-Sayed MH, El Etreby KB, Dunaway E, et al. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine at birth. J Ayub Med Coll Abbottabad 2006 Oct-Dec;18(4):4-9.

22. Lu CY, Ni YH, Chiang BL, Chen PJ, Chang MH, Chang LY, et al. Humoral and cellular immune responses to a hepatitis B vaccine booster 15-18 years after neonatal immunization. J Infect Dis 2008 May;197(10):1419-1426.
24. Saffar MJ, Rezai MS. Long-term antibody response and immunologic memory in children immunized with hepatitis B vaccine at birth. Indian Pediatr 2004 Dec;41(12):1232-1237.

25. Salama II, Sami SM, Salama SI, Rabah TM, El Etreby LA, Abdel Hamid AT, et al. Immune response to second vaccination series of hepatitis B virus among booster dose non-responders. Vaccine 2016 Apr;34(16):1904-1908.

26. Lu S, Ren J, Li Q, Jiang Z, Chen Y, Xu K, et al. Effects of hepatitis B vaccine boosters on anti-HBs-negative children after primary immunization. Hum Vaccin Immunother 2017 Apr;13(4):903-908.

27. Sadeghi Y, Yahyapour V, Poortahmasebi S, Shahmoradi M, Roggendorf H, Karimzadeh S, et al. Clearance of HBV DNA in immunized children born to HBsAg-positive mothers, years after being diagnosed with occult HBV infection. J Viral Hepat 2016;23:282-285.

28. Wang Q, Klenerman P, Semmo N. Significance of anti-HBe alone serological status in clinical practice. Lancet Gastroenterol Hepatol 2017 Feb;2(2):123-134.