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## Original article

# Assessment of hepatitis B virus immune status among hepatitis B virus vaccinated children according to Egyptian regulations

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**Abstract**

**Background::** Vaccination compared to other measures is considered the most efficient and cost-effective strategy against hepatitis B virus (HBV) infection which decreases the incidence of both HBV infection and hepatocellular carcinoma. This study involved 179 children vaccinated against HBV during infancy, and aimed to assess the immune status against HBV among HBV vaccinated children in different age groups. **Methods:** Participants were chosen according to previously settled inclusion and exclusion criteria. Recruited participants' ages ranged from 9 months till 10 years who had received the full routine infancy vaccination course. Participants were divided according to age into three groups: group A ages from 9 months to less than 2 years of age (41 children 22.9%), group B ages from 2 years to less than 5 years (64 children 35.8%), and group C ages from 6 years to 10 years (74 children 41.3%). Serum levels of antiHBs were measured. **Results:** Out of 179 participants, there were 154 children (86%) seroprotected, while 25 children (14%) were not. Moreover, 100% of children under 3 years were seroprotected. Seroprotection rate under 5 years was 95.2%. While in group C seroprotection rate was 73%. Males showed a slightly higher seroprotection rate than females although statically insignificant. **Conclusion:** Universal hepatitis B vaccination shows excellent effectiveness in Egypt. Total seroprotection rate (86%) is higher than in previous local and global studies that may return to the recent implementation of birth dose in Egypt.

## Introduction

Hepatitis B virus (HBV) is clinically important viral infection as the number of infected individuals with it is relatively higher than most of the other viruses. It is considered a one of the major public health problems worldwide; about 30% of people around the world (350-500 million people) have been infected with HBV at some point and show serological evidence of current or past infection [1-3].

The WHO has estimated that approximately 100 million African individuals have chronic HBV infection, and all African countries have an intermediate (2% to 7%) or high (equal or more than 8%) endemicity of chronic HBV infection. Mother-to-child (perinatal) and early childhood transmission the most common modes of transmission in regions of intermediate or high endemicity. The WHO African Regional Committee in November 2014 has endorsed a decision for hepatitis B infection control to reduce the rate of chronic HBV infection to less than 2% in children less than 5 years of age in region by 2020 [4].

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The development of HBV vaccines has greatly reduced the global burden of HBV infection. One hundred eighty-nine countries have adopted universal hepatitis B vaccination strategy by the end of 2018, which has effectively reduced the global incidence of HBV infection in children less than 5 years from 4.7% in the prevaccine era to 1.3% [5]. However, universal hepatitis B vaccination strategy integration in some regions is suboptimal and timely implementation of birth dose vaccine is not adopted in more than half of newborn infants. Optimal and timely adoption of universal hepatitis B vaccination all over the world requires more efforts to surpass the social and economic challenges [5, 6].

The present study aimed to assess the immune status against HBV among HBV vaccinated children in different age groups.

## Method

Approval of medical research institute ethics committee and the medical manager of El-shabti hospital were obtained, signed written informed consent was obtained from all parents of participants. All participants' parents were interviewed, demographic data and full history were taken.

This study involved 179 participants (95 males "53.1%" and 84 females "46.9%") attending the outpatient clinics of El-shabti university hospital. Recruited participants' ages ranged from 9 months till 10 years who had received the full compulsory routine primary vaccination course (full course as combined at 2, 4, and 6 months of age for all participants, in addition to the birth dose as a single within the first 24 hours of life only in participants less than 5 years), and who didn't have any known chronic diseases or immune system problems. Participants were divided according to age into three groups: group A included ages from 9 months till < 2 years of age (41 children 22.9%), group B included ages from 2 years till < 5 years (64 children 35.8%), and group C included ages from 6 years till 10 years (74 children 41.3%).

Sampling period extended from May 2021 to October 2021, a blood sample of 3 ml was withdrawn from each participant, and were centrifugated at 1500 rpm for 10 min to get serum samples and stored at – 20°C until laboratory investigation. Anti HBs levels were measured by using Quantitative anti HBs ELISA method ProClinTM 300 (BEIJING KEWEI CLINICAL DIAGNOSTIC REAGENT INC.) according to manufacture instructions.

**Results of participants were divided to** [7, 8]:

Non-seroprotected: when HBs were < 10 IU/L, the universally recognized cut off value,

Seroprotected: when anti-HBs serological concentration ≥10 IU/L.

**Seroprotected participants were further subdivided into** [9]:

Low level seroprotected when anti-HBs antibody concentrations between 10 and 100 IU/L

High level seroprotected when antiHBs concentrations > 100 IU/L.

## Results

Results of vaccination immune response rates and levels of seroprotection were analyzed in regard to three variables that were age, birth dose administration, as well as sex. Out of 179 participants, there were 154 children having protective levels of anti-HBs which means that total seroprotection rate was (86%) and the percentage of achievement of high level seroprotection (51.9%) versus (48.1%) low level seroprotection in total, while 25 children (14%) unseroprotected. It was observed that 100% of children under 1 year were high level seroprotected, 100% of children under 3 years were seroprotected, majority of them (77.3%) were high level seroprotected.

**A comparison between the three studied groups according to anti-HBs concentrations (IU/L) was carried out:**

There was a statistically significant difference between the three studied groups in regard to seroprotection rate. Group A has the highest seroprotection rate (100%) within the three studied groups as shown in **(table 1)**.

There was no statistically significant difference between group A and group B neither in regard to seroprotection rate nor in regard to levels of seroprotection within the protected participants, while between group A and group C, there was a statistically significant difference in regard to both previous variables. The same result was also found between group B and group C; there was a statistically significant difference in regard to seroprotection rate and levels of seroprotection within the protected participants.

**Another comparison between the two studied sectors (groups A and B versus group C)**

**according to anti-HBs concentrations (IU/L) was carried out:**

There was a strong statistically significant difference between the first sector "groups A and B" and the second sector "group C" as showed in **table (2)**.

**Relation between seroprotection rate and levels of seroprotection (IU/L) in different age categories:**

There was a statistically significant difference in the level of seroprotection in different age categories as showed in **table (3).**

**A comparison between male and female genders according to anti-HBs concentrations (IU/L) was carried out:**

There was no statistically significant difference between males and females in seroprotection rate (*p* = 0.158) as showed in **figures (1&2)**

**Table 1.** Comparison between anti-HBs concentrations (IU/L) among the three studied groups.

| | Total (n = 179) | | Group A (n = 41) | | Group B (n = 64) | | Group C (n = 74) | | χ2 | *p* | Sig. bet. grps. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | | | p1 | p2 | p3 |
| Concentrations (IU/L) | | | | | | | | | | | | | |
| Non seroprotected (<10) | 25 | 14.0 | 0 | 0.0 | 5 | 7.8 | 20 | 27.0 | 19.178* | <0.001* | FEp= 0.154 | <0.001* | 0.003* |
| Total protected | 154 | 86.0 | 41 | 100.0 | 59 | 92.2 | 54 | 73.0 | | | | | |
| Low level seroprotected (10 – 100) | 74 | 48.1 | 11 | 26.8 | 27 | 45.8 | 36 | 66.7 | 15.128* | MCp= 0.001* | 0.055 | <0.001* | 0.025* |
| High level seroprotected (>100) | 0 | 1.9 | 0 | 3.2 | 2 | 4.2 | 8 | 3.3 | | | | | |

χ2: Chi square test FE: Fisher Exact
p: p value for comparing between the studied groups
p1: p value for comparing between Group A and Group B
p2: p value for comparing between Group A and Group C
p3: p value for comparing between Group B and Group C
*: Statistically significant at $p \leq 0.05$
Group A: 9 month – <2 year
Group B: 2 year – <5 year
Group C: 5 year – 10 years

**Table 2.** Comparison between the two studied sectors according to anti-HBs concentrations (IU/L).

| | Total (n = 179) | | Group A + B (n = 105) | | Group C (n = 74) | | χ2 | *p* |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | | |
| Concentrations (IU/L) | | | | | | | | |
| Non seroprotected (<10) | 25 | 14.0 | 5 | 4.8 | 20 | 27.0 | 17.909* | <0.001* |
| Total protected | 154 | 86.0 | 100 | 95.2 | 54 | 73.0 | | |
| Low level seroprotected (10 – 100) | 74 | 48.1 | 38 | 38.0 | 36 | 66.7 | 11.544* | 0.001* |
| High level seroprotected (>100) | 80 | 51.9 | 62 | 62.0 | 18 | 33.3 | | |

χ2: Chi square test
*p*: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$
Group A + B:9 month – <5 year
Group C: 5 year – 10 years

**Table 3.** Relation between seroprotection rate & levels of seroprotection (IU/L) in different age categories.

| | Age (years) | | | | | | | | | | | | | | | | | | | | | | $\chi^2$ (p) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group A | | | | Group B | | | | | | Group C | | | | | | | | | | | | |
| | <1 (n = 8) | | 1 – <2 (n = 33) | | 2 – <3 (n = 23) | | 3 – <4 (n = 20) | | 4 – <5 (n = 21) | | 5 – <6 (n = 13) | | 6 – <7 (n = 14) | | 7 – <8 (n = 11) | | 8 – <9 (n = 14) | | 9 – <10 (n = 12) | | 10 – <11 (n = 10) | | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | |
| **Concentrations (IU/L)** | | | | | | | | | | | | | | | | | | | | | | | |
| Non seroprotected (<10) | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 15.0 | 2 | 9.5 | 4 | 30.8 | 5 | 35.7 | 3 | 27.3 | 1 | 7.1 | 6 | 50.0 | 1 | 10.0 | 31.191* ($^{MC}p$<0.001*) |
| **Total protected (≥10)** | **8** | **100** | **33** | **100** | **23** | **100** | **17** | **85.0** | **19** | **90.5** | **9** | **69.2** | **9** | **64.3** | **8** | **72.7** | **13** | **92.9** | **6** | **50.0** | **9** | **90.0** | |
| Low level seroprotected (10–100) | 0 | 0.0 | 11 | 33.3 | 8 | 34.8 | 12 | 70.6 | 7 | 36.8 | 6 | 66.7 | 5 | 55.6 | 4 | 50.0 | 11 | 84.6 | 4 | 66.7 | 6 | 66.7 | 27.279* ($^{MC}p$=0.002*) |
| High level seroprotected (>100) | 8 | 100 | 22 | 66.7 | 15 | 65.2 | 5 | 29.4 | 12 | 63.2 | 3 | 33.3 | 4 | 44.4 | 4 | 50.0 | 2 | 15.4 | 2 | 33.3 | 3 | 33.3 | |

**Figure 1.** Comparison between Male and Female genders according to anti-HBs concentrations.

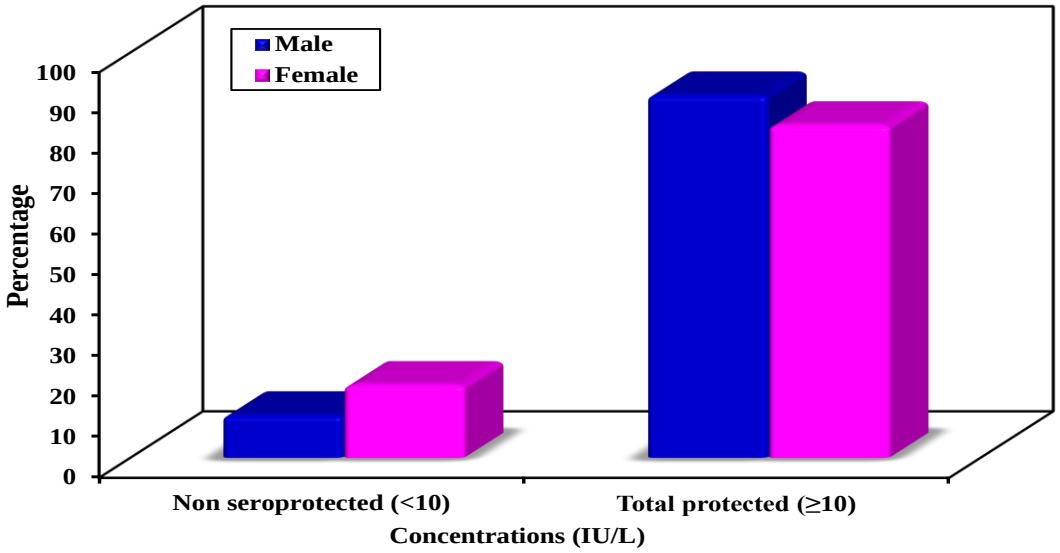
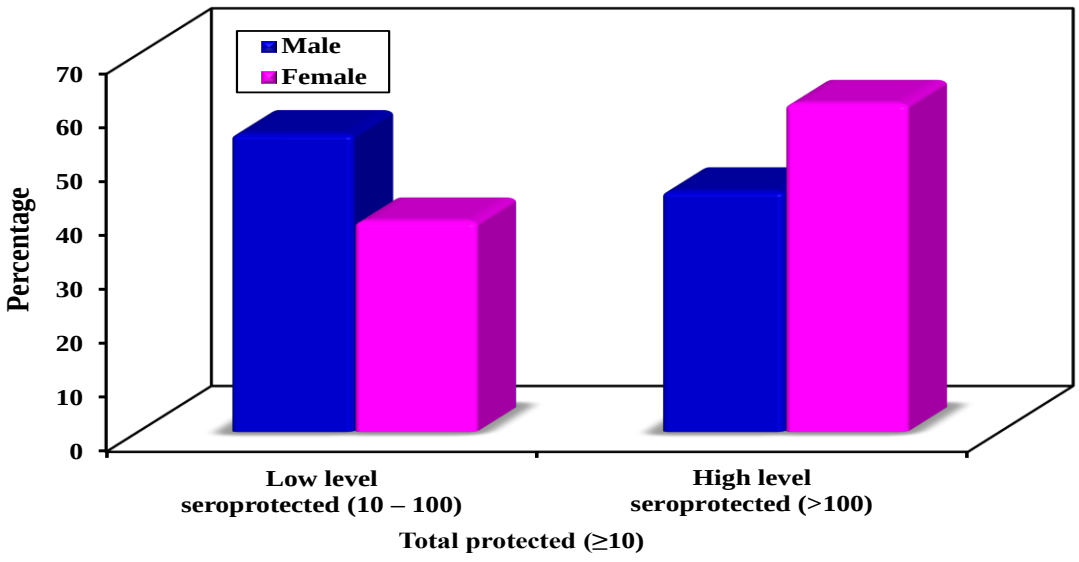



**Figure 2.** Comparison between male and female genders according to levels of seroprotection (IU/L).



**Discussion**

A complete three dose series of vaccination can induce long-term protection which lasts for more than 30 y, but the exact duration of an effective immunologic cellular memory is not well known [5,10,11].

The Egyptian health authorities in October 1992 have adopted the universal infant HBV compulsory vaccination with the recombinant DNA-based vaccine at 2, 4, and 6 months of age into the routine schedule of the national immunization program [12,13], and adopted the birth dose of vaccination regardless of the infection status of the mother in October 2016 as a strategy for minimizing the perinatal transmission from unknown serology, false negative, or delayed serology results of mothers [14, 15]. Nevertheless, the assessment of primary vaccination early and long term sero-protection rate beginning from one to three months after routine vaccination is lacking in Egypt [16].

The present study revealed that the total sero-protection rate among ages ranging from 9 months till 10 years in fully vaccinated children with recombinant HBV vaccine during infancy was 86% (154 children out of 179 participants), 48.3% of them were low level seroprotected while 51.9% of them were high level seroprotected. However, lower percentages were reported among Egyptian children by **Sami et al., 2016, Salama et al., 2014, and, Salama et al., 2020,** (69.8%, 77.35% and, 71.6% respectively) [16-18]. Also, lower percentages were reported in the same age range by **Al-Shamahy et al., 2011** [19], their study revealed that only 54.8% of vaccinated healthy children from 1 to 10 years were seroprotected. Another study in China revealed that the total seroprotection rate in children aged 1 to 15 Y was 60.9% [20]. An Indian multicentric study carried out by **Puliyel et al., 2018** [21] on 2671 children reported total seroprotection occurrence in 70% of the participants. A recent Indian study, 68.86% was the overall seroprotection rate of 122 children aged 1 to 10 years after vaccination [22].

The present study showed that, all members (100%) of group A (9 month to less than 2 years) developed a protective level of antibodies, 73.2% of them were high level seroprotected and only 26.8% were low level seroprotected. Also, the results showed that 100% of children under 1 year were high level seroprotected. On the contrary, **Puliyel et al., 2018** [21] reported that seroprotection rate in the first year of age was 82%, among 2671 Indian children, and a lower seroprotection rate of 66.7% was previously reported within the first year of age by **Al-Shamahy et al., 2011** [19].

In group B (2 years to less than 5 years) in the current study, there were 5 children (7.8%) of this group members didn't have protective anti HBs concentrations, while the seroprotection rate in this group was 92.2%. Meanwhile percentage of high level seroprotection was declined from 73.2% in group A to 54.2% in group B. Also, the seroprotection rate under the age of 3 years was100% majority of them (77.3%) were high level seroprotected, similar results in regard to seroprotection rate under the age of 3 years were reported by **Kumar et al., 2021** [22]. In the current study, approximately two thirds of seroprotected children under 4 years were high level seroprotected.

In the present study, we observed that the failure to achieve seroprotection rate in group C (5 year to 10 years) was 27% (20/74 children) as 20 children of group C members failed to achieve seroprotection in a response to vaccination. Total seroprotection rate within group C was 73%, majority of them (66.7%; "36/54 children") have low antibody levels, and only 33.3% "18/54 children" of group members have high antibody levels.

Moreover, in the current study, in the same age group (group C), at the age categories from 8 to 10 Y, odd results were observed as there was an elevation in seroprotection rate at the ages of eight and ten years, which reached 92.9% and 90 respectively, while at the age of 9 years had the lowest rate of seroprotection (50%) within group C, and also over the whole of the three studied groups "A, B, and C". This variation in seroprotection rates may be due to uneven sample sizes in each category.

This decrease in rate and level of seroprotection in group C compared to groups A and B in the current study may be logically a result to advance in years of age in group C as age is recognized as a well-established risk factor for gradual depletion of anti-HBs concentrations [18, 22,23], or it may be a result to birth dose administration in groups A and B which is supposed to be the root cause for elevation in the rates and levels of seroprotection in these two groups (A and B).

Higher rates of seroprotection were reported by **Rezaei et al., 2014** [24] in a similar study in Iran, where researchers reported relatively

higher seroprotection rate (78%) in children between 5 to 10 years of age, and it further decreased to 74% in 10 years of age. On the other hand, Egyptian studies within the same age group [5 Y to 10 Y]; lower seroprotection rates (66.1%, 56.8%, and 60.8%) were reported respectively by **Salama et al., 2014** [16, 17, 18]. Also, in a study carried out in India by **Aggarwal et al., 2014** [23], 53% of children aged from 5 to11 years have protective anti HBs levels. Another study in South Korea reported that the seroprotection rate after 7 years of age was 50% [25].

In the present study, comparing the seroprotection rate and seroprotection levels among the three studied groups, a statistically significant difference was found ($p < 0.001$, and $p < 0.001$). Also, by comparing seroprotection rates and levels, between each of A and B, A and C, and B and C groups, the results revealed that, there was no statistically significant difference between group A and group B regarding seroprotection rates ($p = 0.154$), or seroprotection levels within the protected participants ($p = 0.055$). There was a statistically significant difference in both the seroprotection rate and in the levels of seroprotection between group A and group C, as the seroprotection rate in group A was 100%, while in group C it was only 73% ($p < 0.001$). Furthermore, as regard to seroprotection levels within the protected participants, there was a strong statistically significant difference as high level seroprotection rate in group A was 73.2% while in group C it was only 33.3% ($p < 0.001$).

Comparing group B and group C, there was a statistically significant difference as regard to both the seroprotection rate and the seroprotection levels within the protected participants, as the rate of non seroprotection in group B was 7.8% while in group C it was 27%, and the seroprotection rate in group B was 92.2% while in group C it was only 73% ($p < 0.003$). Also, as regard to the levels of seroprotection within the protected participants, there was a statistically significant difference in high seroprotection levels between group B and group C ($p < 0.025$) (54.2% in group B while in group C it was only 33.3%).

In the current study, when meditating on the children who are less than 5 years of age (the HBV birth dose vaccinated sector "groups A and B"), it was observed that there was a high immune response to HBV vaccination in these two groups; as more than 95% of participants were seroprotected, and the majority of them (59%) had high level seroprotection, versus a seroprotection rate of 73% and a high level of seroprotection of only 33.3% in the group above 5 years (group C who didn't receive the birth dose).

A lower seroprotection rates in the age groups less than 5 years of age were observed in Egypt by **Salama et al., 2014** [18], **Sami et al., 2016** [17], and **Salama et al., 2020** [16]; (88.6%, 82.8%, and 82.4% respectively). Also, **Al-Shamahy et al., 2011** [19], and **Alssamei et al., 2017** [26] have reported lower seroprotection rates within the similar age groups (54.8%, and 72.2%, respectively). A similar study in Iran, researchers reported that seroprotection rate in children less than 5 year after vaccination was 88% [24]. Also, Puliyel et al 2018 [21] reported that the anti HBs levels declined from 82% at the first year to 47% before the fifth years.

In the current study, although males showed a slightly higher seroprotection rate than females, yet there was no statistically significant difference in the rate of immune response ($p = 0.158$), or in seroprotection levels ($p = 0.046$) between both genders, which is similar to the report of **Sami et al., 2016** [17], and **Salama et al., 2020** [16] where seroprotection rate in males (54.0%) was slightly higher than females (52.8%). Also, **Al-Shamahy et al., 2011** [19], **Lee et al., 2017** [25], and **Kumar et al., 2021** [22] reported that males have a slightly higher seroprotection rate than females (57.4 vs 51%, 35.3% vs. 32.1%, and 76% vs 55.6%) respectively, that is in contrast to the report of **Rezaei et al., 2014** [24] from Iran, and **He et al., 2016** [20] from China have reported higher seroprotection rates in female children than in male children. On the other hand, other Iranian and Brazilian studies have reported no serological differences between male and female children [27, 28].

**Strengths and weaknesses**

To the best of our knowledge, there were no previous local Egyptian studies comparing the birth dose administered sector and the birth dose non-administered sector. In this study, we compared the two studied sectors; the first sector ["groups A and B" (9 month to less than 5 year)] that represents the HBV vaccination birth dose administered population versus the second sector [group C (5 Y to 10 Y)]; the HBV vaccination birth dose non-administered sector. The results revealed that, there

was a strong statistically significant difference between the rate of seroprotection and non-seroprotection, as well as between high and low levels of seroprotection between the two sectors. Also, the risk for non-seroprotection in group C is five folds higher than in both groups A and B. This confirms the hypothesis that birth dose enhanced both the vaccine immune response rate and higher levels of seroprotection.

**Recommendations**

From the present study we recommend the following (Un answered questions):

- Further studies are required on a larger number of children in different age groups to evaluate the HBV vaccination effect in different age groups. Also, further studies are required to assess the HBV vaccination immune response in other pediatric special subgroups as immunocompromised subgroups.
- It is recommended to assess the anti-HBs concentrations at the age of 9 months or in the first year of life after completion of the infancy compulsory routine vaccination schedule in all Egyptian HBV vaccinated children for the continuation of the birth dose.

**Conclusion**

From the present study we concluded the following:

Universal hepatitis B vaccination shows excellent effectiveness in Egypt. Total present seroprotection rate (86%) is higher than in previous local and global studies and also the present study found high percentage of achievement of high level seroprotection (51.9%) versus (48.1%) low level seroprotection that may return to the implementation of birth dose in Egypt which enhanced the HBV vaccination immune response and also enhanced higher levels of seroprotection.

**Competing interest**

We declare that we have no conflict of interest.

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