

Prevalence of seroprotective antibodies against hepatitis B virus in immunized children in a community hospital

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ABSTRACT

Background: The potential problem of hepatitis B (HB) vaccination is the declining levels of antibody to HB surface (anti-HBs) antigen causing concern regarding its protective efficacy. **Objectives:** The objectives were to study the rate of seroconversion of children of age 1–12 years immunized against HB in a community hospital and to assess the status of seroprotection in those who have attained seroconversion. **Materials and Methods:** Blood samples of children between 1 and 12 years of age immunized against HB were tested for the presence and titer of the protective antibody anti-HBs. Results: A total of 196 children were included in this study. The seroconversion rate in this study were 91.8%. The overall seroprotection rate (>10 mIU/ml) were 80%. The seroprotection in the age group 1–3 years were 90%, 4–6 years was 80%, 7–9 years were 64%, and 10–12 years were 46.7%. As the age advances, seroprotective titers decrease significantly ($p=0.001$). Children who had procured vaccination through private institutions were found to have better seroprotection than children who received vaccination through government sector ($p=0.001$). In this study, the median anti-HBs levels were 169.75 mIU/ml. **Conclusion:** In this study, the overall seroprotection rate was lower than that reported in various EPI program though it was acceptable in the 1–3 years age group. The seroprotection rates decreased exponentially with age. The GMT was well within the recommended level.

Key words: Anti-hepatitis B surface, Seroprotection, Seroconversion

Hepatitis B viral (HBV) infection is one of the major liver diseases of mankind and is a serious global public health problem because of its high morbidity and mortality [1]. The biomarkers associated with HBV infection helps in the identification of disease, stratification of chronic hepatitis and therapy [2]. Acute HBV infection in children is mostly asymptomatic; although, some may present with fatigue, anorexia, malaise, arthralgia, or skin lesions. Extrahepatic manifestations associated with HBV infection are polyarteritis nodosa, glomerulonephritis, and aplastic anemia. Most of them recover, but certain children go into complications such as acute liver failure, chronic HB, cirrhosis, and hepatocellular carcinoma which are associated with high mortality [3,4].

Treatment of acute HBV infection is largely supportive. Treatment of chronic HBV infection is in evolution; no one drug currently achieves consistent, complete eradication of the virus. Therefore, prevention is the mainstay of treatment [3]. Apart from screening blood donors for HB, following universal precautions while handling blood products, passive immunization with HB immunoglobulin, active immunization with HB vaccine is the most effective means of preventing the infection. There has been a significant decrease in the prevalence of HBV infection in countries which have adopted the immunization program diligently.

However, immunization is also not 100% effective due to various reasons. Despite immunization with three doses of HB vaccine in infancy, 5–10% of children do not show seroconversion [5]. Among babies born to HBs antigen (HBsAg) positive mothers who receive active and passive immunization at birth, 5–10% still develops HB infection [6]. Formation of vaccine escapes mutants which will decrease the efficacy of existing vaccine and eventually cause evasion of the vaccine.

The recombinant HBV yeast derived vaccine has a number of limitations that justify the development of new HBV vaccines such as the need for multiple doses, lack of long-lasting immunity, inability to induce both humoral and cellular immunity against the antigenic targets of major genotypes of virus, and incomplete protection in vaccinees where a group of non-responders does exist and vaccine is therapeutically ineffective [7]. Hence, this study was designed to assess the prevalence of seroprotective antibodies against HBV in previously immunized children attending a community hospital.

MATERIALS AND METHODS

This cross-sectional study was done in a tertiary care center serving the urban and rural population around Chennai from

January 2016 to April 2016. Approval from hospital authority, scientific committee and ethics committee were taken before starting the study. The sample size was calculated using the following formula and was estimated to be 196.

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

$$n = \frac{2.576 \times 2.576 \times 0.92(1-0.92)}{0.05^2}$$

$$n = 196$$

Where n = required sample size, the expected proportion or seroconversion and seroprotection rate (p) = 0.92, t = confidence level at 99% (standard value of 2.576), m = margin of error at 1% (standard value of 0.05) at desired confidence level of 99%.

This study was conducted on children between 1 and 12 years of age attending the pediatric outpatient department. Children, who had a complete record of vaccination duly, endorsed in vaccination cards, were included in the study. Children <1 year of age and children with incomplete vaccination or without vaccination cards were excluded. A questionnaire was used and details regarding the child’s age, gender, date of birth, data concerning HB vaccination status, and vaccination cards were revised for date and dose intervals of HB vaccine, family history of HB and if mother is HBsAg positive, details of active and passive immunization given in the newborn period and follow-up visits were filled up and analyzed.

After obtaining informed consent, about 2 ml of blood sample was withdrawn from each participant. Serum samples were aliquoted into labeled tubes and stored at 2°to8°C until laboratory examination. After blood sample collections, plasma samples were separated and tested for anti-HBs using enzyme-linked immune sorbent assay following the manufacturer’s protocol. As per the WHO standards, anti-HBs antibody titers of >10 mIU/ml were taken as cutoff for protective level and samples showing titers <10 mIU/ml were considered nonprotective. Samples showing anti-HBs titers above 1mIU/ml were considered to have seroconverted.

The test principle was based on antigen sandwich enzyme immunoassay. Patient’s serum or plasma sample was added to the microcells together with HBsAg conjugated to Horseradish peroxidase (HRP-Conjugate). In case of presence of anti-HBs in the sample, the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody, and during incubation, the specific immunocomplex formed is captured on the solid phase. After washing to remove the sample and unbound HRP-Conjugates, chromogen solutions containing tetramethylbenzidine and urea peroxide are added to the wells. In the presence of the antigen-antibody-antigen (HRP) “sandwich” complex, the colorless chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample, respectively. The sensitivity of the test was 100%, and specificity was 98.8%.

Data were entered in Microsoft Excel sheet, and statistical analysis was done using SPSS (Statistical Package for the Social Sciences) version 19.0. Chi-square tests were done to compare the categorical variables seroconversion rate, seroprotection rate among the immunized children. The study population was divided into four age groups: 1–3 years, 4–6 years, 7–9 years, and 10–12 years. Anti-HBs levels were classified as <10 mIU/ml, 11–100 mIU/ml, 101–500 mIU/ml, and >500 mIU/ml and were studied among the four individual age groups and results were interpreted. The median values of anti-HBs titers were calculated using Mann–Whitney U-test. For continuous measurements of anti-HBs levels, the comparison between age groups were done using Mann–Whitney U-test and Fischer’s Test. p<0.05 was considered statistically significant (Fig. 1).

RESULTS

A total of 196 children between 1 and 12 years of age who had completed all the three doses of HB vaccine series were enrolled in the study. In this study, the majority of the children were falling under 1–3 years age group (Table 1). Of 196 children studied 104 (53%) were males and 92 (46.9%) were females. 185 (94.38%) children received vaccination through private institutions and 11 (5.61%) children received through government hospitals. Of 196 children, 185 children had taken birth, 6 weeks, 6-month schedule whereas 11 children had taken 6 weeks, 10 weeks, and 14-week schedule (Table 2). In our study, none of the children had a history of jaundice in family members and none of the children were born to HBsAg positive mothers

Of 196 children, 180 children showed seroconversion with an anti-HBs level above 1 mIU/ml, and the seroconversion rate was 91.8%. The remaining 16 children did not seroconvert, and the percentage of non seroconversion was

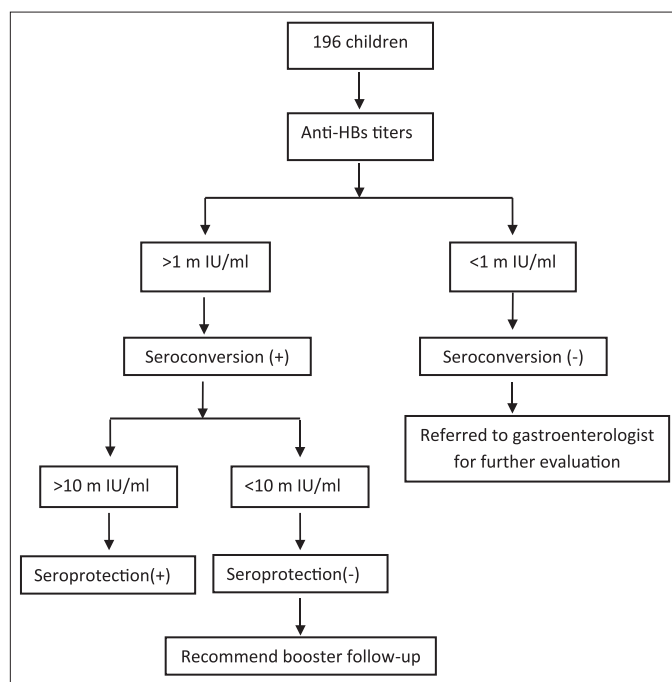


Figure 1: Study flow chart

Table 1: Distribution of seroprotection among four individual age groups

Seroprotection	Age group				Total	p
	1–3 years (%)	4–6 years (%)	7–9 years (%)	10–12 years (%)		
Yes	81 (90.0)	40 (80)	16 (64)	7 (46.7)	144 (80.0)	<0.001
No	9 (10.0)	10 (20)	9 (36)	8 (53.3)	36 (20.0)	
Total	90 (100)	50 (100)	25 (100)	15 (100)	180 (100)	

Table 2: Distribution of seroprotection based on HBV immunization schedule

Seroprotection	Immunization schedule		Total (%)	p
	0, 6 weeks, 6 months (%)	6 weeks, 10 weeks, 14 weeks (%)		
Yes	141 (82.9)	3 (30)	144 (80.0)	0.001
No	29 (17.1)	7 (70)	36 (20.0)	
Total	170 (100)	10 (100)	180 (100)	

HBV: Hepatitis B viral

8.2%. Of 180 seroconverted children, 36 (20%) had anti-HBs levels <10 mIU/ml (non-protective level) while 144 (80%) had anti-HBs levels above 10 mIU/ml (protective level). As the age advances, the seroprotective rate decreases as shown in Table 3. The seroprotective rates of children among 1–3 years age group were 90%, 4–6 years age group were 80%, 7–9 years age group were 64%, and 10–12 years age group were 46.7%. The difference was statistically significant (p<0.001).

Among the study population, males had a seroprotection rate of 81.6% while females had a seroprotection rate of 78% and the difference was not statistically significant. Of 144 seroprotected children, 141 children had taken 0, 6 weeks, and 6 months schedule whereas 3 had taken 6 weeks, 10 weeks, and 14 weeks schedule. Of 36 children who are not seroprotected, 29 had taken 0, 6 weeks, 6 months schedule and 7 had taken 6 weeks, 10 weeks, and 14 weeks schedule. The seroprotective rates were superior in children who were vaccinated with 0, 6 weeks, and 6 months schedule than in children with 6 weeks, 10 weeks, and 14 weeks (p<0.001) as shown in Table 2.

Of 185 children who received vaccination through government hospitals, 43 children had anti-HBs titers <10 mIU/ml and 142 children had anti-HBs titers>10 mIU/ml. Of 11 children vaccinated in private sector, 8 had anti-HBs titers<10 mIU/ml, and 3 children had anti-HBs titers>10 mIU/ml. Seroprotection rates were higher in children vaccinated through private institutions than government hospitals (p<0.001). The overall median anti-HBs levels were 169.75 mIU/ml, and the interquartile range was 8.9 and 384.05 mIU/ml. The median anti-HBs levels among individual age groups are shown in Table 4. With the advancing age, the median anti-HBs levels tend to decrease significantly (p<0.001).

DISCUSSION

HB is a global health problem with variable prevalence in different parts of the world. Various studies conducted in India showed that the prevalence of hepatitis as 2.4% whereas in tribal population, the figures are much higher (15.9%). It is estimated that India hosts about 40 million chronic HB carriers, which is almost 4% of the entire population. Age at the time of acquisition plays an

Table 3: Median anti-HBs titers in each age group

Age (years)	Number of children	Anti-Hbs levels (mIU/ml)		p [§]
		Median	IQR	
1–3	97	333.5	(85.41,422.75)	<0.001
4–6	54	63.2	(9.31,343.05)	
7–9	28	17.12	(3.51,105.56)	
10–12	17	7.69	(2.5,31.04)	
Overall	196	169.75	(8.90,382.05)	

§Mann–Whitney U-test. HBs: Hepatitis B surface, IQR: Interquartile range

important role in predicting the carrier state. HB acquired during infancy is responsible for a very high risk of cirrhosis in later life. HB is a preventable disease and vaccination at birth can eradicate the disease if vaccine strategy is effectively implemented.

In our study, 145 of 196 children had anti-HBs >10 mIU/ml and 51 children had anti-HBs <10 mIU/ml. These results were comparable to a study done by Afzal *et al.* [6], where 133 children had anti-HBs >10 mIU/ml and 61 had anti-HBs <10 mIU/ml. In our study, 180 children showed seroconversion (anti-HBs>1 mIU/ml), and seroconversion rate was 91.8%. These results were comparable with study done by Khan in Bangladesh [8]. The seroconversion rate was 92.4% in males, and 89.1% in females and these results were compared with results of the study by Zamani *et al.* [9].

Of 180 children who had seroconverted, 36 (20%) had non-protective levels while 144 (73.97%) had protective levels of anti-HBs. The seroprotection rate in our study was 80%, and it correlates with the results of study done by Saffar and Rezai [10] and Sugunan *et al.* [11] As the age advances, the seroprotective rate decreases and this was comparable with the results of various other studies [12–17]. The GMT of anti-HBs was 7.69 mIU/ml which is very less compared to the GMT of studies done by Saffar *et al.* [10] In our study, seroprotection after 5 and 10 years were 64.7% and 46.7% which is lesser than the study reports of Gilca *et al.* [18] (88.2% and 86.4%, respectively). This difference may be due to the differences in the type of vaccine used, amount of the antigen delivered, and the population immunized.

A similar study was done in Turkey for children between age group 2 and 12 years by Alavian [19]. They reported that the rate

Table 4: Distribution of serum levels of anti-HBs among individual age groups

Anti-HBs level (mIU/ml)	Age group				Total	p
	1–3 years (%)	4–6 years (%)	7–9 years (%)	10–12 years (%)		
<10	15 (29.4)	14 (27.5)	12 (23.5)	10 (19.6)	51	<0.001
11–100	10 (23.3)	19 (44.2)	9 (20.9)	5 (11.6)	43	
101–500	68 (69.4)	21 (21.4)	7 (7.1)	2 (2)	98	
500	4 (100)	-	-	-	4	
Total	97 (49.5)	54 (27.6)	28 (14.3)	17 (8.7)	196	

HBs: Hepatitis B surface

of persistent protective level of anti-HBs titers varied from 33% to 79%, 5 years after vaccination and their results were consistent with our study results. In another study done by Eldesoky *et al.* [20] in Egypt, the persistence of protective antibody titer was 53% in vaccinated children aged 6–10 years. In our study, the seroprotection rates were 81.6% and 78% in males and females, respectively. This was consistent with the study reports of el-sawy and Mohamed [21].

In our study, there was a significant decline in anti-HBs titers as the age advances ($p < 0.001$). These results were consistent with the study results of Hosseine *et al.* [12] in Iranian children and El-Sayed *et al.* [22] in Egyptian children. The seroprotective rates were superior in children who took birth, 6 weeks, 6 months schedule than in children who took 6 weeks, 10 weeks, and 14 weeks ($p < 0.001$). However, this was different from the study done by Wouters *et al.* [23]. They compared the immunogenicity of schedules administered at 0, 1, 4 months and 0, 1, 6 months among commercial sex workers and found equal efficacy between both schedules.

In our study, parental awareness about the vaccine was good. A study conducted in Vellore showed that the parental awareness of vaccine availability was 71% and knowledge about the vaccine was better for HBV vaccine than other vaccines [24]. Afzal *et al.* [6] showed that all children who received private procured HB vaccine achieved protected antibody levels and the difference between the vaccines procured from public sector and private sector was statistically significant ($p = 0.028$). These results were similar to our study results.

The overall median anti-HBs level in our study was 169.75 mIU/ml, which is lower than the mean GMT of anti-HBs levels in children aged 1–15 years (232.64 mIU/ml) in a study done by Hosseine *et al.* [12]. With the advancing age, the median anti-HBs levels tend to decrease significantly ($p < 0.001$). These results are comparable to a study conducted by Aghakhani *et al.* [25] in children aged 8 months to 15 years. They showed that the mean anti-HBs titer declined with post-vaccination time (to 66 mIU/ml in 1 year, 60 mIU/ml in 5 years, and 40 mIU/ml in 10 years to 37 mIU/ml in 15 years after vaccination). In another study by Dahifar *et al.*, the GMT of anti-HBs 5 years after vaccination decreased to 153 mIU/ml [26].

Our study also has some limitations. First, the sample size was small. Second, we could not check the HBsAg levels, HB core antibody levels and liver function tests for children who did not show seroconversion due to limited resources and funding.

Although we obtained significant association for variables in relation to place of immunization and immunization schedule, we believe that studies with larger sample sizes would be required to further validate our results. We also suggest that children who have low titers should be followed up for breakthrough infection and occurrence of chronic infection which may occur if there is a lapse of immunological memory. Children who have not seroconverted should be evaluated in detail including all HBV biomarkers, and if HBsAg is negative, they should be revaccinated with three doses of HB vaccine and rechecked for seroprotective status 4 weeks after the last dose.

CONCLUSION

Overall, seroprotection in our study was lower than the previously reported rates; however, it was acceptable in children between 1 and 3 years of age. There was an exponential drop in seroprotection rates with increasing age; however, this does not indicate the need for a booster because of the immunological memory. The dosing of 0, 1, and 6 months schedule showed a better seroprotection endorsing the view that it is better to immunize neonates soon after birth.

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