Ten-year serological follow up of hepatitis B vaccine recipients

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Aim: To determine long-term persistence of antibodies to hepatitis B surface antigen (anti-HBs) after vaccination against hepatitis B. **Methods:** Thirty-four laboratory workers received hepatitis B vaccine in 1989 in a 0-1-6 month vaccination schedule. Group A (n=16) received a booster at 3 years after vaccination whereas Group B (n=18) did not. Anti-HBs was quantitated at 1 month and 1, 2, 3, 5, 6 and 8 years post-vaccination. **Results:** At eight-year follow up, 10 of 15 subjects in Group A and 3 of 16 in Group B had protective levels of anti-HBs; in addition, two and four subjects, respectively, had detectable anti-HBs though below protective levels. At ten years, 9/15 and 3/16 were anti-HBs positive in Groups A and B, respectively. One subject in each group had rise in anti-HBs titer at 6-year follow up but both of them tested negative for IgG antibodies to hepatitis B core antigen (anti-HBc). A booster dose at 10 years to anti-HBs negative subjects led to an anamestic response in 3/4 and 8/10 persons in Groups A and B, respectively. **Conclusion:** Immunological memory after vaccination against hepatitis B is maintained for at least 10 years. [*Indian J Gastroenterol* 2000;19:168-171]

Key words: Hepatitis B antibody, vaccination

A majority of hepatitis B virus (HBV) infections in India are acquired horizontally, in early childhood and adulthood.[1] Childhood infections frequently result in carrier state.[2] In Indian conditions, therefore, an ideal vaccine against HBV would be one that protects against childhood infections as well as against horizontal infection in adulthood.

Persistence of antibodies to hepatitis B surface antigen (anti-HBs) over several years after vaccination has been shown in several studies.[3,4,5] Even among individuals whose anti-HBs titers have fallen below protective levels, an anamnestic immune response to HBV can be elicited.[6] Such long-term data are not available from India. Seroresponse to HBV vaccine and persistence of anti-HBs may depend on factors such as age at vaccination, obesity, smoking habits[7] and perhaps varying immune response of different populations.

In India, no guidelines for post-vaccination screening and the need, if any, for booster doses are available. It is hence important to obtain data regarding the dynamics of anti-HBs levels over a long period of time in the Indian population. Our study reports the status of anti-HBs titers over a period of ten years after vaccination.

Methods

Forty-four laboratory workers (mean age 37.6 [SD 8.8] years; 33 men) in frequent contact with blood and blood products received three doses of HBV vaccine (20 mg per dose; *Engerix-B;* SmithKline Biologicals, Bangalore) at 0, 1 and 6 months, intramuscularly in the deltoid region; the last dose was given in 1989.[8] Thirty-four of these vaccinees (mean age 37.3 [SD 8.3] years; 23 men) consented to participate in a long-term serological follow-up study; 3 of them were primary nonresponders. The study was cleared by the institutional ethics committee. Informed consent was obtained from each participant.

The vaccinees were followed up at one month after the third dose of primary vaccination and at 2, 3, 5, 6, 8 and 10 years. At every follow up, history of jaundice, other sickness, parenteral therapy, hospitalization and blood transfusion in the intervening period was noted. Blood samples were drawn and sera stored at -20°C till tested. Sixteen vaccinees (Group A) who were thought to be at greater risk of exposure to HBV, being field workers in direct contact with patients, were administered a booster dose of HBV vaccine (20 mg IM; *Engerix-B*) at 3 years. The remaining 18 (Group B) did not receive any booster dose.

At 10 years (April 1999), blood samples of 15 vaccinees in Group A and 16 in Group B were collected; one vaccinee in Group B was lost to follow up. Four anti-HBs negative subjects in Group A and 11 in Group B were administered a 20 mg booster *(Engerix-B)*, intramuscularly in the deltoid region. Blood samples from 14 of them (4 from Group A, 10 from Group B) were collected 2 weeks after administration of booster.

Serological tests

Blood samples were tested for hepatitis B surface antigen (HBsAg) using an in-house enzyme immunoassay (EIA) employing mouse monoclonal anti-HBs to coat the solid phase and goat anti-HBs IgG conjugated with horse radish peroxidase. The sensitivity of this sandwich EIA was 0.5 ng/mL and specificity 100% as compared to commercial EIAs. Antibodies to hepatitis B core antigen (anti-HBc) were tested using a commercial EIA (*Corzyme;* Abbott Laboratories, USA). Anti-HBs was tested using radio immune assay (*AusAB;* Abbott

Laboratories, USA). All samples were tested undiluted and in dilutions of 1:100, 1:1000 and 1:10,000, and 1:100,000 if required, as per the manufacturer's instructions. Anti-HBs was quantitated using RIA units. If the ratio of counts per minute of sample to that of negative control (S/N) was equal to or greater than 10, anti-HBs titer was considered to be protective; S/N 2.1-10 was positive but non protective, and S/N <2.1 was negative.[9] Ten-year follow-up samples were tested qualitatively using EIA (*AUSAB–EIA;* Abbott Laboratories, USA).

One vaccinee developed acute viral hepatitis at 18 months post-vaccination. Several samples, i.e., 6 months prior, and 1, 7, 15 and 30 days following onset of jaundice were tested for IgM and IgG antibodies against hepatitis E virus (anti-HEV),[10] HBsAg and IgM anti-HBc (*Corzyme-M;* Abbott Laboratories, USA).

Statistical methods

Geometric mean titers (GMT) were calculated for both the groups at each time point. Samples testing negative were also included for this purpose; these samples were assigned an arbitrary titer of 1. Student's *t* test was used to compare log GMTs at different time points. Regression analysis was carried out by the least squares methods, using a software (SPSS, version 6.1.4) and anti-HBs titers for a period of 16-20 years were extrapolated.

To determine the dependence of antibody titers at 8-years follow up on the initial peak titers, Pearson's correlation coefficient was calculated between log titers post third dose and log titers at 8-years follow up for Group B. For Group A, Pearson's correlation coefficient was calculated between log titers post third dose of primary vaccination and log titers at 5-years follow up to determine dependence of boosting effect on initial titer.

Results

The non-responder from Group A who suffered from hepatitis 18 months after vaccination was found to be IgM anti-HEV reactive in the acute-phase serum. The sample taken 6 months before the episode of hepatitis was non-reactive for IgG anti-HEV. Seroconversion to IgG anti HEV was also recorded. The samples were negative for HBsAg, IgM anti-HBc and IgG anti-HBc and remained so throughout the study period.

The remaining vaccinees remained IgG anti-HBc negative during the study period of 10 years and did not develop jaundice.

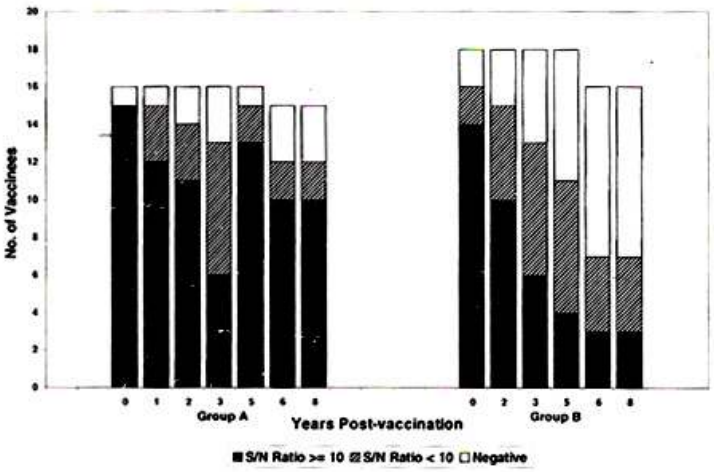

Fig 1: Anti-HBs levels in hepatitis B vaccine recipients over 8 years

Group A (boosted at 3 years)

One month after the third dose, 15 of 16 vaccinees had protective levels of anti-HBs and one vaccinee tested negative for anti-HBs (Fig 1). At three years post-vaccination, six vaccinees had S/N ³10, seven had S/N below 10, and three were anti-HBs negative. At 5 years post-vaccination (2 years after the booster), 13 persons had S/N ³10, two had S/N <10 and one person was negative. Six years after vaccination, one vaccinee had a 14-fold rise in anti-HBs titers as compared to the 5-year titer but IgG anti-HBc was non-reactive; this subject was excluded from further analysis. At both 6 and 8 years post primary vaccination; 10 of 15 vaccinees had protective levels of anti-HBs; in two persons S/N was <10 and three tested negative.

Group B (not boosted at 3 years)

One month after the third dose of vaccine, 14 of 18 vaccinees had protective anti-HBs titers, whereas two had S/N <10 and two were anti-HBs negative (Fig 1). At 3-year follow up, six vaccinees had S/N ³10, seven had S/N <10 and five had no detectable anti-HBs. Five years after vaccination, four vaccinees had S/N ratio ³10, 7 had S/N <10 and 7 were negative for anti-HBs. At six-year follow up, one vaccinee had a 10-fold rise in anti-HBs titer as compared to anti-HBs levels at 5 years but IgG anti-HBc was negative; this subject was excluded from further analysis. Another vaccinee was lost to follow up. At both 6 and 8 years, three vaccinees had protective anti-HBs levels; four had S/N <10 and 9 tested negative.

Anti-HBs positivity status at 10 years

Nine of 15 vaccinees in Group A and 3 of 16 in Group B tested anti-HBs positive. Four and 10 vaccinees in

Groups A and B, respectively, who tested negative received a booster; of these, 3 and eight, respectively, developed anti-HBs with S/N >10. The vaccinee in Group A who remained anti-HBs negative had been a non responder after primary vaccination but had shown weak anti-HBs positivity (S/N <10) after the 3-year booster.

The two vaccinees in Group B who did not respond to booster dose had S/N <10 after primary vaccination and had become anti-HBs negative after 2 and 8 years, respectively.

Kinetics of antibody decline

A ten-fold decline in GMT of anti-HBs was observed at one year (Group A) and two years (Group B) post vaccination (Table). Subsequent decrease in GMT was gradual over the next 8 years in Group B. In Group A a significant ($p<0.001$) rise in GMT was observed after administration of a booster dose at 3 years, followed by a gradual decline.

One of the vaccinees from Group A who was a non-responder after primary vaccination seroconverted to anti-HBs on receiving a booster at 3 years. At 5 years, he was anti-HBs reactive with S/N <10. At 6 years, anti-HBs could not be detected. When boosted at 10 years after vaccination he did not become anti-HBs positive. Two non-responders in Group B remained negative for anti-HBs throughout the follow-up period; when a booster was administered at 10 years, one of them became anti-HBs positive with S/N <10.

For Group A, regression analysis was carried out for the post-booster period. The equation best suited for this was found to be

$$\text{Log (GMT)} = 1.95 + 74.68\ e^{-\text{time}}$$

$p=0.04$ (one-tailed t test, d.f.=1)

For Group B, a similar regression analysis was carried out taking all time points after primary vaccination into consideration. The equation suited for this was

$$\text{Log (GMT)} = 0.39 + 1.25\ e^{-\text{time}}$$

$p=0.00007$ (t test, d.f.=4)

Table: Kinetics of anti-HBs antibody decline over 8 years

Time after last dose of primary vaccination	Geometric mean titer (range) Group A	Group B
1 month	102.4 (1-8600)	33.8 (1-3200)
1 year	10.8 (1-300)	Not done
2 years	10.0 (1-512)	3.6 (1-320)
3 years	4.1 (1-512)	3.3 (1-160)
5 years	291.0 (1-32000)	2.7* (1-32)
6 years	124.2 (1-12500)	2.4* (1-32)
8 years	99.7 (1-10000)	2.0* (1-32)

*$p<0.05$ as compared to Group A

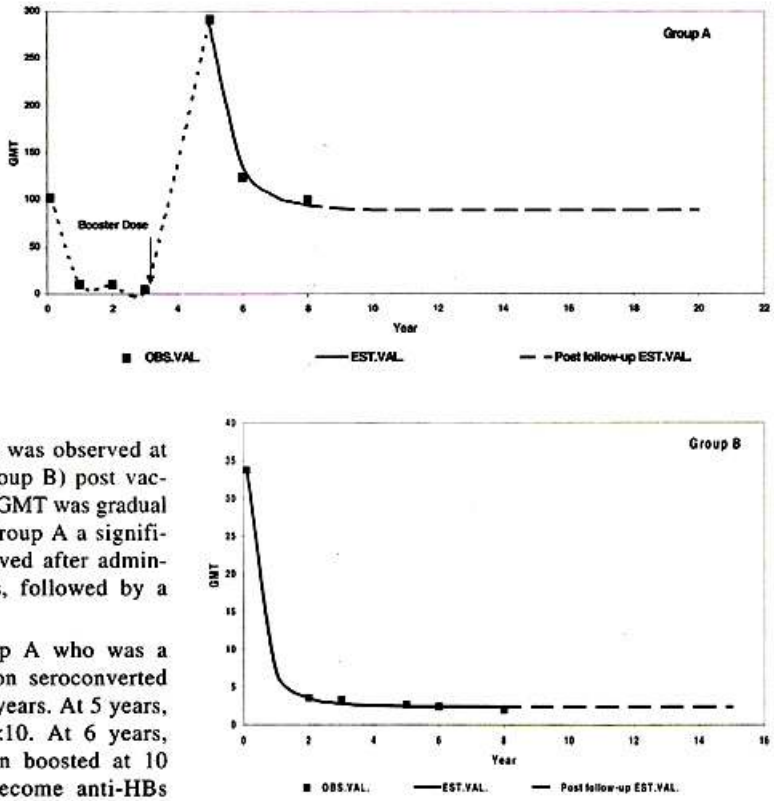

Fig 2: Dynamics of anti-HBs decline in Groups A (top) and B

Extrapolation of the curves (Fig 2a) indicated that in Group A, anti-HBs GMT could be expected to decline to 88.7 at 15 years, after which the decline was estimated to be negligible. In Group B, further decline in GMT below the levels reached at 8 years was estimated to be negligible (Fig 2b).

In Group B, anti-HBs titers at 8 years were dependent on the peak antibody titers ($r=0.66$, $p<0.05$). Similarly, in Group A, the boosting effect of the booster dose was dependent on the initial titers ($r=0.88$, $p<0.01$).

Discussion

Numerous studies have been conducted to determine the duration of protection provided by HBV vaccines.[3-6] Ours is the first long-term follow up of Indians vaccinated against HBV.

In agreement with others,[5] our study shows that antibody titer at 8 years has direct relation to peak antibody levels. We have earlier shown that after primary vaccination a significantly lower anti-HBs titer was elicited among persons aged >30 years.[8] Anti-HBs titer may also depend upon other factors like obesity, smoking habits[7] and perhaps varying immunological

responses in different populations. Studies[11] have shown that an additional dose of vaccine, i.e., a regime of 0-1-2 and 12 months is likely to elicit a higher peak anti-HBs titer in comparison to the regime employed in the present study. Our study indicates that those receiving an additional dose at three years had higher titer during follow up. Hence, at least in populations likely to have a lower response, a four–dose schedule may be beneficial.

About 19% of unboosted vaccinees showed evidence of protective anti-HBs titers 8 years after vaccination, whereas 25% had anti-HBs but not in protective titers. In a cohort of Yupik Eskimos,[4] at 8 years after vaccination, nearly 75% in the age group 20-40 years and 55% in the above-50-years age group maintained protective levels of anti-HBs. Among Indians, therefore, anti-HBs decline seems to be more rapid. Antibody was, however, estimated to last for at least 15 years. In the group receiving an additional dose of vaccine, anti-HBs was estimated to last for at least 20 years. Further follow-up data will be needed to substantiate these estimates.

In the present cohort, two individuals with protective anti-HBs titers had an anamnestic response to HBV more than five years after vaccination. Since anti-HBc was consistently absent in both of them, replication of HBV was ruled out. This suggests that, on exposure to HBV, vaccinated individuals are protected from disease for a duration of at least five years.

Our study has also shown that 8/10 persons became anti-HBs positive after booster at 10 years, suggesting that immunological memory is maintained.

In spite of some studies demonstrating a four-fold more frequent seroconversion to anti-HBc among vaccinees with anti-HBs titer below 10,[9] other groups have documented that retention of circulating anti-HBs in protective titer may not be an important criterion for protection against hepatitis B infection.[6] Immunological memory may last up to 12 years after vaccination among persons in whom serum anti-HBs is not detectable.[6]

It is known that a significant proportion of HBV infections in India occur among young children and adults.[1] HBV vaccine conferred immunological memory for at least 10 years, and estimates indicate that anti-HBs may last for as long as 20 years after four doses of vaccine. We therefore feel that even if persons are vaccinated at an early age in India, protection in infancy as well as from subsequent horizontal transmission could be achieved without administering a late booster.

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