Will infant hepatitis B vaccination protect into adulthood? Extended Canadian experience after a 2, 4, 6 month immunization schedule

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Cover title: HBV protection 10-16 years after vaccination at 2,4,6 months

Running head: HBV protection after infant vaccination

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ABSTRACT

Introduction

Hepatitis B virus (HBV) vaccination programs generally target infants to prevent chronic HBV infection and/or pre-adolescents to reduce transmission in adulthood. To assess whether infant HBV immunization can potentially accomplish both objectives we measured residual immunity 10-16 years afterward in Canadian children.

Methods

A prospective, parallel group, single center study enrolled adolescents given HBV vaccine at about 2, 4, 6 months of age. Exclusion criteria included prior HBV infection and additional vaccinations. At follow-up anti-HBs testing participants were 10-11 or 15-16 years old; those possibly lacking protection (<12 mIU/mL anti-HBs with assay used) were challenged with HBV vaccine to assess immune memory-based responsiveness.

Results

137 tested participants were 10-11 and 213 were 15-16 years old, respectively; none had evidence of prior HBV infection. At baseline, 78.1% of younger and 64.3% of older participants had <12 mIU/mL anti-HBs (p=0.006) and were challenged with vaccine: 103/106 (97.2%) younger and 123/135 (91.1%) older participants developed ≥12 mIU/mL anti-HBs (p=0.06), with GMC of 590 (95%CI 473, 737) and 319 mIU/mL (95% CI 229, 445)(p=0.004), respectively. Immune memory loss may have occurred in 3 younger (2.2%) and 12 older children (5.6%) (p=0.06) who were non-responsive to first but not second vaccine challenge.

Conclusions

After HBV vaccination at 2, 4, 6 months of age, most adolescents had little or no residual antibody but nearly all responded to HBV challenge, confirming immune memory persistence. However, anamnestic responses were weaker in 15-16 year olds and lost in some. Booster responses in 10-11 year olds were vigorous in comparison. ~~booster vaccination at this age would likely extend measurable protection into adulthood.~~ Extended evaluation of protection is warranted.

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INTRODUCTION

Recombinant hepatitis B vaccines provide an effective means to prevent infections caused by the hepatitis B virus (HBV), including the long-term complications of cirrhosis and liver cancer (1,2). Since the World Health Organization called for global use of HBV vaccines in 1992 (2) at least 183 countries have implemented vaccination programs (2). Countries with high endemic rates of HBV infection that were early adopters of routine vaccination have reported marked rate reductions in acute and chronic infection (3–7). Follow-up studies extending several decades have confirmed that immunized individuals enjoy prolonged protection (5,8–10). Breakthrough infections occur occasionally after child and adult vaccination but more frequently after infant vaccination, usually without establishment of chronic infection (5,8,11–13).

HBV vaccination programs routinely target infants or adolescents or both. The goal of infant programs is to reduce the high rates of chronic infection commonly associated with infection in early life (2,14) while the goal of adolescent programs is to reduce peak rates of acute infection seen in early-to-mid adulthood, when progression to chronic infection is infrequent (2). Available evidence confirms the success of both types of programs (10,14–16). However, an important remaining question is whether infant vaccination can accomplish both goals, including prevention of significant disease well into adulthood. A challenge to evaluating program outcomes is the diversity of schedules recommended for infant HBV immunization. WHO guidelines recommend a birth dose followed by 2-3 doses co-administered with other early childhood vaccines according to the local schedule (2). In the United States, CDC guidelines also recommend a birth dose for all, followed by doses at 1-2 and 6-18 months or at 2,4,6 months when using a combination vaccine containing HBV antigen (17). Greater diversity is evident in the European Union, where many countries limit the birth dose to infants born to infected women, with 3-4 subsequent doses administered in a variety of schedules (0,1,6 months; 2,3,4 months; 2,4,6 months etc). Some countries substantially delay the final dose after 2-3 initial doses, with recommended ages varying between 10 and 15 months of age (18).

In Canada, British Columbia (BC) has provided universal infant HBV vaccination since 2001, after a pilot program in Vancouver in 1998-2000. As BC already had an established program to detect infected pregnant women and administer HBV vaccine to their newborns (0,1,6 month schedule), a decision was made to provide the vaccine to low-risk infants at 2,4,6 months of age, conveniently co-administered with routine infant vaccinations. BC also provided HBV vaccine routinely to adolescents in a school-based program, established in 1992. Graduates of

the provincial infant program reached the age at which adolescent immunizations had been given (10-11 years) in 2012, leading to cessation of the adolescent program and reliance on infant vaccination for extended protection. We undertook to study whether the assumption of durable protection was well founded when HBV vaccinations are provided at 2,4,6 months of age. In a meta-analysis of 46 studies (15) examining the persistence of anti-HBs after infant vaccination, all but one study provided doses at about 0,1,6 months of age, consistent with the WHO/CDC recommendations. Detailed analysis of variation in the interval between doses 2 and 3 confirmed that wider separation (≥4 months) provided superior long-term immunogenicity (15), presumably because the delayed third dose boosted final responses. The compressed 2,4,6 month schedule might prime infants less effectively, with a reduced series response potentially reducing the duration of protection. This possibility was important to address as compressed schedules have become increasingly widely used to take advantage of combination infant vaccines that include HBV vaccine. An additional factor that might reduce the duration of ~~sero~~protection is the absence of natural boosting in low HBV endemicity countries like Canada and the USA (19). This is particularly relevant because natural boosting might have affected the rates of antibody persistence reported from countries with higher HBV endemicity that predominated in the above-mentioned meta-analysis (15).

The immune response to HBV vaccination includes production of antibody against HBV surface antigen (anti-HBs) (2,11,20) as well as T and B cell responses (12,21,22). A vaccinated individual is considered to have ~~sero~~protection if post-vaccination serum antibody values are ≥10 mIU/mL(2). This correlates best with protection against chronic infection but does not predict 100% protection against acute or subclinical infection (11,14). Anti-HBs concentrations decline following vaccination, possibly falling below the protective or detection threshold, but immune memory often persists, enabling rapid recall of a protective antibody response following natural exposure or booster vaccination (20,23). Given the lengthy incubation period of HBV infection (2,24), an anamnestic response is likely to provide effective ~~sero~~protection against chronic infection. However, antibody persistence and the anamnestic response are variable among individuals and both are proportional to the antibody response obtained after primary vaccination (13,15,23).

The stimulus to conduct this study was the lack of data on long-term persistence of antibody following the compressed 2, 4, 6 month infant HBV immunization schedule, especially from a low prevalence region without the potential for natural boosting.

METHODS

The study was approved by the University of British Columbia Research Ethics Board. A list of randomly chosen infant vaccinees from two age groups (10-11 and 15-16 year olds) was provided by the Vancouver Coastal Health Authority from an immunization registry database, with permission. Only those with 3 HBV immunization dates conforming to the recommended infant schedule were included. Another list of potential participants was generated using a database of our previous study participants who had consented to future contact. In this instance, HBV immunization schedule compliance was verified from parent or provider records. The older age group was the first to receive infant HBV vaccine in Vancouver and the younger age group mimicked the age at which pre-adolescents were previously offered HBV vaccine, as a benchmark for comparisons. Families were sent an invitation letter describing the study, then telephoned to offer further information. Preliminary eligibility screening was conducted by telephone.

Participants were from the Vancouver area and had to have been born in BC, participated in the routine infant vaccination program at about 2, 4 and 6 months of age and have a record of those immunizations. None had serology testing following series completion. Exclusion criteria included having been immunized on an alternative HBV infant schedule or with additional doses of HBV vaccine; having evidence of prior HBV infection; suffering from conditions associated with immunosuppression during childhood; and receipt of blood products within 3 months. For this study, compliance with the routine schedule required that the first dose of the primary HBV series should have been received between 6 and 12 weeks of age, the third dose prior to 36 weeks of age, and the interval between second and third doses was not to exceed 13 weeks. Recombinant HBV vaccine (Recombivax, Merck Frosst Canada) was routinely administered to both age groups as infants. A proportion of the older participants likely received adult doses of vaccine, although this was not known at study onset (more information follows in Discussion).

At participants' first visit, eligibility was reviewed and informed consent and/or assent was obtained. A baseline blood sample was obtained to determine anti-HBs concentration and to test for HBsAg and anti-HBc to rule out prior infection (eligibility condition). Hepatitis B tests were performed at the British Columbia Centre for Disease Control, the reference laboratory for the province. The assay used was an FDA-approved CLIA-based commercial assay (ADVIA Centaur anti-HBs2 assay, Siemens Healthcare Diagnostics inc, Tarrytown, NY),that expressed results in milli-International Units per milliliter (mIU/mL), with a threshold of ≥10 mIU/mL for

protection and a lower limit of detection of 3.1 mIU/mL. Since the assay has a margin of error of 10±2 mIU/mL, we followed the manufacturer's recommendation to use values ≥12 mIU/mL as more certain to indicate protection without repeated testing of samples.

Participants with anti-HBs titers ≥12mIU/mL were advised that they were protected and exited the study. Those with titers <12mIU/mL were invited to return for challenge vaccination with a standard pediatric dose of HBV vaccine (10 mcg Engerix B, GSK, as currently used in the BC program). All doses of vaccine were from the same lot. Participants were monitored for any immediate allergic reaction for 15 minutes after vaccination and encouraged to report any later adverse events of concern.

Continuing participants returned 28 days (±7 days) after challenge vaccination when eligibility and adverse events were reviewed and blood for anti-HBs measurement was obtained. Participants found to have anti-HBs ≥12 mIU/mL exited the study; others were asked to return for a second challenge dose of HBV vaccine, administered as before. These few participants returned 28 days (±7 days) later when blood was obtained to determine their anti-HBs status. Participants with persistently undetectable anti-HBs were possibly primary non-responders, who might also have failed to respond to their primary series. These individuals were informed of their results and advised to seek additional doses of HBV vaccine. The best evidence of loss of immune memory was provided by participants who lacked anti-HBs before and after initial challenge but who developed ≥12 mIU/mL after the second challenge, ruling out inability to respond.

Statistical Plan and Power Calculations

Based on a meta-analysis of similar follow-up studies (15), we estimated that about 30% of younger and 60% of older participants would lack seroprotection (anti-HBs <12 mIU/mL). We estimated that 25-30% of older non-protected participants would not have a recall response to challenge, representing 15-20% overall of that study population. To detect a rate of 15% with a precision of ±0.05 and 95% confidence intervals, we determined that 220 individuals would need to be tested, allowing for a 10% drop-out rate. Amongst the younger challenge dose recipients, we projected that 10% would not have a recall response, representing an overall susceptibility rate of 3%. For a precision of ±0.03 and 95% confidence intervals, we determined that 140 participants would need to be assessed, allowing for a 10% drop out rate.

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute, North Carolina) where the PROC FREQ and PROC MEANS were used to compute antibody threshold rates, geometric mean antibody concentration (GMC) and corresponding 95% confidence intervals. P-values were computed for proportional data using Fisher's exact test while GMCs were compared by t test. All available data were included in the analyses in the event of participant withdrawal after testing.

RESULTS

Study Population and Participation

Of over 3200 individuals invited by mail to participate, 710 proved not to have a current address or telephone number or could not speak English. Of 1276 individuals who were interviewed by telephone, 917 declined to participate. When the target of 359 volunteers was reached, recruitment efforts ceased, with 1214 mailed invitations not pursued. Subsequent participant flow is outlined in Figure 1. Six enrollees were deemed ineligible after being enrolled as a result of late disclosure of neonatal HBV vaccination (n=3), ineligible medical condition (n=2) or additional doses of HBV vaccine (n=1). After these 6 exclusions and 3 early consent withdrawals, 350 participants (194 males and 156 females) underwent baseline testing. Thereafter, 3 participants withdrew consent (citing needle aversion) and one was lost to follow-up, for a net protocol completion rate of 96% (346/359).

~~The average age of study participants was 10.6 years for the younger cohort and 16.0 years for the older cohort~~. Table 1 summarizes the demographic characteristics of study participants. Males predominated in the older group (59:41 ratio) (p=0.08, compared with the younger group). Ethnicity distribution was similar in both age groups, reflecting local population diversity. Health conditions reported by participants were predominantly minor or intermittent ailments, including asthma, skin conditions and anxiety disorders. Mean ages at starting (9 weeks) and completing (6.3 months) infant HBV immunization were uniform between age groups and matched the recommended schedule.

Baseline Serology

Baseline test results were available for all 350 participants (Figure 1). None had detectable anti-HBc or HBs antigen present. Anti-HBs concentrations <12 mIU/mL were present in 107/137 (78.1%) younger participants and 137/213 (64.3%) older participants, a significant rate difference (p=0.006) (Table 2). Protection rates did not vary significantly by sex. Among participants with antibody concentrations below 12 mIU/mL, 51.1% of younger and 36.2% of older participants had undetectable values (<3.1 mIU/mL). The geometric mean anti-HBs concentrations in both age groups were low at baseline but somewhat higher in the older group (Table 2). No significant difference in baseline GMC was noted between males and females in either age group (data not shown).

First Challenge Responses

Of participants with <12 mIU/mL anti-HBs at baseline, 106 of the younger and 135 of the older participants returned for HBV vaccine challenge (Figure 1). No adverse events were documented post-vaccination. All but one younger participant returned for the follow-up blood test. Post-challenge, anti-HBs values ≥12 mIU/mL were present in 97.1% of younger participants and 91.1% of older participants (p=0.06) (Table 2). Most (91.4% in the younger group and 79.7% in the older group) (p=0.01) had robust responses to challenge, with anti-HBs ≥100 mIU/mL. A significantly greater proportion of younger than older participants developed post-challenge antibody responses ≥1000 mIU/mL (p=0.02). Furthermore, amongst participants with undetectable anti-HBs at baseline, most of the younger group responded strongly to challenge dosing compared to a lower proportion of the older group (Figure 2). Post-challenge GMC in the younger group was significantly greater than in the older group (Table 2). No significant difference in post-challenge GMC was noted between males and females in either age group.

Second Challenge Responses

Participants with anti-HBs <12 mIU/mL after the first challenge dose (n=15) all returned for a second challenge. No adverse events were documented post-vaccination. All 3 rechallenged participants in the younger group (100%) and 9/12 in the older group (75%) developed protective anti-HBs concentrations afterward (Table 2). The response in these participants was less robust than in those who responded to the first challenge, with only 1 of 3 younger and 2 of

9 (22%) older participants developing anti-HBs ≥100 mIU/mL. The GMC of older group responders was just 6% of the GMC of their peers who responded to a first challenge (20 vs 319 mIU/mL) (Table 2). Taken together, this response pattern is consistent with loss of immune memory after infant vaccination and an early primary response to vaccine challenge. Three older group participants (1.4% overall) lacked detectable antibody after both challenges and likely represented primary non-responders.

DISCUSSION

This study is one of the first and the largest long-term evaluation of immunity after infant HBV vaccination at 2, 4, 6 months of age. While this schedule is convenient and favors use of HBV-containing combination vaccines, we found antibody concentrations 10 years later were substantially lower than reported after the 0,1,6 month schedule. Compared with results of a meta-analysis (15) of 45 studies conducted 10 years after HBV vaccination at 0,1,6 months of age when only 30% of children had anti-HBs concentrations <10 mIU/mL, 78% of 10-11 year olds that we studied had values <12 mIU/mL, suggesting less durable antibody responses after the 2, 4, 6 month schedule. However, neither study included response measurements from infancy to address rates of antibody decay. Lack of natural boosting may have contributed to the low observed anti-HBs concentrations in our study, as in a study from the USA (19). However, despite the relative lack of serum antibodies, none of the younger participants in the current study had evidence of prior HBV infection and nearly all had a recall response to challenge dosing, even those who had no detectable antibody at baseline. The observations indicate that an anamnestic response can be elicited in this group as a result of persistent immune memory, consistent with ongoing protection from infection.

Among the15-16 year olds studied, we estimated that 60% would have anti-HBs concentrations <12 mIU/mL at baseline based on the above meta-analysis (15) and found that to be the case in 64%. However, this result was puzzling as the younger cohort had a higher proportion of individuals with <12 mIU/mL anti-HBs after a shorter interval from prior vaccination. Unknown to the investigators at the outset of this study, many of the older participants likely received adult doses of HBV vaccine (Recombivax, Merck) for their primary series, for lack of an available pediatric formulation at the time (pediatric dosage was recommended but providers were urged not to waste half-doses in opened “adult” vials; actual dosages administered were not recorded so the proportion receiving adult vs. pediatric dosages is unknown). By the time the younger

cohort was born, pediatric dose formats were available. Prior studies of infant HBV vaccination indicate that persistence of anti-HBs is proportional to the primary series dosage received (8, 10-15), which likely explains the higher proportion of individuals with ≥12 mIU/mL anti-HBs in the older than younger cohort.

Despite the frequent absence of HBV antibody levels ≥12 mIU/mL in both age groups, the great majority of participants had an anamnestic response to challenge dosing reflecting ongoing immune memory and likely protection. Fewer older than younger participants responded to challenge dosing, consistent with the literature (15). Despite the infant dosage advantage in the older group, a number of observations point to declining response capacity with increasing age. The 15-16 year olds had a less robust response to challenge dosing when compared to the younger group, developing a GMC about half that of younger participants. Similarly, older participants were less likely to respond with anti-HBs concentrations ≥100 mIU/mL, compared to the younger group (p=0.01), particularly those without detectable anti-HBs beforehand (Figure 2). Responses ≥100 mIU/mL were used by several authors to characterize an anamnestic response in adolescents and adults. (23, 25–27).

We found that 2.2% of younger and 5.6% of older participants (p=0.06) had <12 mIU/mL anti-HBs at baseline and were non-responsive to initial vaccine challenge, suggesting lack of immune memory and protection. Most of these individuals responded to a second challenge, demonstrating a capacity to recognize HBs antigen but the observed antibody responses were weak suggesting new primary responses, as can develop in about one-third of naïve vaccinees after a single dose (28). Persistent non-responsiveness to HBV vaccine was absent among younger participants and present in only 3 older participants (1.4%) consistent with either primary non-responsiveness (29) or a low responder phenotype (30). In the meta-analysis of follow-up studies after infant HBV immunization (15), the modeled rate of persistent protection (including demonstrated immune memory) after 10 years was approximately 95% with adequate dosing and 0,1,6 month schedule compliance and approximately 90% after 15 years, with similar conditions. We observed similar protection rates, speaking against a notable difference in duration of protection with HBV dosing at 2, 4, 6 months of age.

This study had several design strengths. Recruitment was community based and participants were generally reflective of the local population. Compliance of participants with the study protocol was excellent, with only 3.6% attrition. Adherence to the infant schedule was verified from provider records, with limited deviation allowed. A single lot of HBV vaccine was used for

challenges and laboratory testing was standardized in a reference laboratory. Using a 28-day sampling interval following challenge vaccination maximized the potential to detect immune responsiveness (23).

The principal weakness of the study was the limited ability of the challenge vaccination procedure to accurately identify immune memory and anamnestic responses. Other mechanisms might also contribute to protection, including cellular and herd immunity. Strong anti-HBs responses were readily characterized as anamnestic but the methodology used did not allow discrimination between weak anamnestic responses and weak primary responses in those who had lost immune memory. Measurement of anti-HBs responses sooner after challenge would have aided recognition of anamnestic responses while measurement of IgM antibody responses to HBs antigen might have identified primary responses but such an assay was not available. Consequently, given that 20% of the older participants had limited responses to initial challenge (<100 mIU/mL), we might have substantially under-estimated the proportion who had lost immune memory as all post-challenge responses ≥12 mIU/mL were interpreted as anamnestic, although about one-third of naïve vaccinees can develop anti-HBs ≥10 mIU/mL after a single dose (28). In reality, both anamnestic and rapid primary responses might contribute to protection against chronic HBV infection, making the distinction moot. Breakthrough infections were not encountered as evidence of loss of protection but were not expected in an environment of low endemicity and high population immunity from long-standing immunization programs. Other weaknesses included the vaccine dosage difference between the age groups in infancy (described above) with undefined variability in dosing of the older group, which precluded modeling of age-related changes in immunity. The vaccine used for primary vaccinations was Recombivax while the challenge vaccine provided for our study was Engerix B. Challenge responses might have been affected by this mismatch but mixed vaccine primary immunization schedules had no effect on final responses in other studies (31,32) . Documentation of individual responses to primary HBV immunization in infancy was not available. Departing from the usual seroprotection threshold (12 vs 10 mIU/mL) as required by the available immunoassay did not materially affect study results as few results (e.g. 3.7% of baseline samples) fell within this range

This study indicates that British Columbia's HBV vaccination program for low risk infants at 2, 4, 6 months of age provides satisfactory protection to mid-adolescence. The question of whether infant HBV vaccination can protect into adulthood lacks a definite answer but mounting evidence points to differences in duration of protection after immunization in early infancy

versus later ages. Substantial evidence already exists that individuals immunized as adolescents or young adults enjoy long-term protection, documented 15 to 30 years following vaccination (10,33), well into the period of heightened risk for adults. No booster vaccination is recommended in this context (2,16). In contrast, multiple follow-up studies (15) after infant immunization show a progressive loss of detectable immunity (seroprotection and immune memory) in up to 15-20% of individuals by 15 years after vaccination, with potential development of breakthrough infections (13) depending upon exposure risks. Follow-up studies after infant vaccination in The Gambia (13) demonstrated increasing rates of breakthrough infection (identified by presence of anti-HBc) among young adults, reaching 30% among 25-29 year olds although few had chronic infection. While the Gambian experience is exceptional, reflecting high HBV exposure risks and possible population differences, it demonstrates the potential for loss of protection after infant immunization. ~~Loss of immune memory after primary tetanus toxoid immunization in infancy has also been documented among adolescents in the absence of any childhood booster doses (former34).~~

Comparison of long-term outcomes after infant and adolescent HBV vaccination highlights the differences between them. For example, pre-adolescents who were given HBV vaccination in a prospective study (33) in Quebec, Canada, and tested 10 years later (age 19) had a residual seroprotection rate (anti-HBs ≥10 mIU/mL) of 86% (61% with ≥1000 mIU/mL) and a GMC of 169 mIU/mL. For the 10-11 year olds in our study vaccinated as infants, the residual seroprotection rate was 4-fold lower at 21.9% (none with >1000 mIU/mL) and the GMC was 40-fold lower at 4.2 mIU/mL. Assuming that the HBV booster response reflects residual B cell response capacity, it is noteworthy that the post-booster GMC of 19 year olds in Quebec was 32,477 mIU/mL, whereas that of 15-16 year olds in BC was 100-fold lower, at 319 mIU/mL. All teens in the Quebec study had received a booster but only non-seroprotected teens (64% of the total) in the present study were boosted, so the results are not directly comparable.

Given such differences, one can reasonably argue that sufficient uncertainty exists about long-term protection after infant vaccination to warrant reconsideration of the infant priming regimen, favoring schedules with more doses or antigen, use of adjuvants or more delayed final doses, as with the current schedules in Belgium and Germany (doses at 2,3,4 and 11-15 months, in addition to a birth dose for high risk infants) (18). While WHO does not currently recommend a booster dose for any age group (2,16), booster vaccination in adolescence might eventually prove useful as a means to reinforce and extend protection in those immunized as infants. Whether a booster dose could extend protection of adults immunized as infants to better

resemble that of adults immunized as adolescents remains an important unanswered question. The fact that substantial differences exist in measures of residual protection among teenagers after infant or adolescent HBV vaccination warrants close ongoing scrutiny of whether important differences will emerge in long-term protection, with or without booster vaccination.

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Conflict of Interest Statement

The authors report no financial conflicts of interests relevant to this study.

Legends for Figures

Figure 1: Study Participation Summary

Figure 2: Post-challenge anti-HBs responses of participants with baseline values ≤3.1 mIU/mL (undetectable) or 3.2-11.9 mIU/mL (sub-protective), showing separately the responses of 10-11 and 15-16 year olds

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