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Active Immunization to Prevent *De Novo* Hepatitis B Virus Infection in Pediatric Live Donor Liver Recipients

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This study aims to evaluate the efficacy of HBV vaccination as an alternative preventive measure against *de novo* HBV infection in pediatric living donor liver transplantation (LDLT). Sixty recipients were enrolled in this study. Thirty received grafts from anti-HBc(+) donors, and another 30 received grafts from anti-HBc(−) donors. HBV vaccine was given pretransplant to every candidate. Posttransplant, lamivudine was routinely given to recipients receiving anti-HBc(+) grafts for about 2 years. Forty-seven (78%) recipients achieved high levels of anti-HBs titer (>1000 IU/L). Two (3.3%) recipients developed *de novo* HBV infection where one received an anti-HBc(−) graft and another received an anti-HBc(+) graft. Both recipients were in the lower anti-HBs titer group (<1000 IU/L). The incidence of *de novo* HBV infection was significantly higher in the lower titer group (15.4% vs. 0%, $p = 0.04$). The median follow-up period was 51 months in recipients with anti-HBc(−) grafts and 57 months in those with anti-HBc(+) grafts. Active immunization is an effective method to prevent *de novo* HBV infection. It can result in high levels of anti-HBs titer (>1000 IU/L) which may prevent *de novo* HBV infection in pediatric patients with efficient primary vaccination undergoing LDLT.

Key words: *De novo* hepatitis B virus infection, immunization, living donor liver transplantation, pediatric

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Introduction

The acquisition of hepatitis B virus (HBV) infection after liver transplantation in recipients who are hepatitis B surface antigen (HBsAg)-negative pretransplant has been recognized (1). The incidence of *de novo* HBV among patients who received anti-HBc(−) grafts is low with incidences ranging from 0% to 1.7% (2–5). However, the use of liver grafts from anti-HBc(+) donors carries a 38–100% risk of *de novo* HBV infection in naïve recipients without prophylaxis (2,4–6). Hence, some centers have suggested to exclude these grafts from anti-HBc(+) donors or to limit its use in selected recipients. This strategy is not practical in endemic areas for HBV infection, such as Taiwan, where 15–20% of the general population is HBsAg(+) and approximately 80% of the adults are anti-HBc(+) (7).

Several strategies have been recommended to prevent *de novo* HBV infection in a recipient who has received a graft from an anti-HBc(+) donor. Hepatitis B immunoglobin (HBIG) and/or lamivudine have been used for prophylaxis (8–11). Our previous report on lamivudine monotherapy refers to this regimen as simple and effective in preventing *de novo* HBV in recipients using anti-HBc(+) grafts. The incidence of *de novo* HBV decreased from 37.5% in recipients without prophylaxis to 0% in recipients with prophylaxis. However, the use of HBIG is expensive and inconvenient, and lamivudine alone can give rise to mutant strains.

The difference between innate and exogenously administered antibody is unknown. The acquisition of immunity through active immunization is preferred if both are equally effective. Active immunization has been suggested against *de novo* HBV. However, these few studies usually combined HBIG and vaccination in limited cases and are with short follow-up. Moreover, the protective level of anti-HBs still remains unclear in patients after liver transplantation.

The aims of this present study are (1) to evaluate the usefulness of active immunization in preventing *de novo* HBV infection in pediatric living donor liver transplantation (LDLT) and (2) to determine the appropriate level of titer to prevent *de novo* HBV after liver transplantation.

Patients and Methods

Between March 1998 and September 2002, 68 consecutive pediatric LDLT were performed in Chang Gung Memorial Hospital-Kaohsiung Medical

Table 1: Demographics and pretransplant hepatitis B profiles based on donor anti-HBc status

Characteristic	Anti-HBc(−)	Anti-HBc(+)
Patient number	30	30
Age (year)	1.7 (0.5–8.8)	1.6 (0.6–14.4)
Gender (F/M)	15/15	12/18
Diagnosis		
Biliary atresia	26	22
Neonatal hepatitis	2	5
Glycogen storage disease	2	2
Alagille syndrome		1
Preoperative status		
Child A	1	4
Child B	17	16
Child C	12	10
Preoperative profiles		
Anti-HBs(+)	100%	100%
Anti-HBc(+)	3 (10%)	5 (16.7%)
Donor anti-HBs	18 (60%)	30 (100%)

Center. Eight recipients were excluded. These were two mortalities (<1-year follow-up), two foreign recipients and four other recipients who were also included in another protocol. Sixty recipients were prospectively enrolled in this study. Thirty recipients received grafts from anti-HBc(+) donors and another 30 received grafts from anti-HBc(−) donors. The recipient demographic characteristics, clinical profile and status of donor anti-HBc serology were shown in Table 1. The surgical techniques and perioperative care were described in detail previously (12,13). All transplantations were ABO compatible. The immunosuppression protocol consisted of cyclosporine, steroid and azathioprine or mycophenolate mofetil (which was used when more potent immunosuppression was required, or for its renal and calcineurin inhibitor sparing benefits). Steroid was withdrawn 6 months posttransplant if no acute cellular rejection occurred (14,15).

Pretransplant, all donors and recipients were tested for HBV serologic markers and antibody using radioimmunoassay (Abbott Laboratories Diagnostics Division, Abbott Park, IL). Almost every pediatric transplant candidate has received three doses of HBV vaccine as a result of a nationwide vaccination program launched in 1984 in Taiwan (16). Every recipient received a booster HBV vaccine (20 μg, Engerix-B, Smithkline Beecham Biologicals, Belgium) before transplant while undergoing evaluation. The donors were also vaccinated if their anti-HBs titers were negative or low before donation.

The protocol for preventing *de novo* HBV infection included lamivudine monoprophylaxis, which was to be given for approximately 2 years posttransplant (4 mg/kg/day, GlaxoWellcome, Middlesex, UK) for recipients (whose anti-HBs were <1000 IU/L) receiving grafts from anti-HBc(+) donors. The reasons for the 2-year period lamivudine use included the potential side effect of its prolonged use such as developing mutant strains and the recipients becoming less immunocompromised as immunosuppressants were tapered when they are likely to achieve protective titers of anti-HBs through booster vaccination. Prophylaxis was not given in recipients who received grafts from anti-HBc(−) donors. Upon steroid withdrawal, sequential HBV vaccinations were administrated to the recipients during subsequent out-patient clinic follow-up at 1–3 months interval to achieve an anti-HBs titer level >1000 IU/L. Recipients who had acquired higher anti-HBs titers or developed acute cellular rejection requiring stronger immunosuppression did not receive vaccination. Hepatitis marker, anti-HBs titers and serum HBV DNA (Digene Hybrid Capture Assay, Digene Corp., MD) were routinely tested every 3 months in recipients who received lamivudine; whereas, recipients not receiving lamivudine were tested every 6 months or more frequently as the need arises if abnormal liver function tests ensued.

De novo HBV infection was defined as HBsAg seropositivity occurring in two consecutive tests in the recipient posttransplant. Once positive for HBsAg, HBV-DNA was extracted from the HBsAg-positive sera, and was amplified by polymerase chain reaction followed by direct sequencing of the nucleotide sequences encoding the *a* determinant of HBsAg (aa 116–160) using a dye terminator cycle sequencing quick start kit (Beckman Coulter, Fullerton, CA). *De novo* HBV was treated with lamivudine, and adefovir dipivoxil (GlaxoSmithKline Inc., Mississauga, Canada) was added if a mutant strain was identified.

All continuous data were expressed as median with range. Nominal data were compared using the chi-square test or Fisher's exact test as appropriate. Significance was considered with p value at <0.05.

Results

Forty-three (72%) recipients were positive for anti-HBs at the initial evaluation for liver transplantation. After pretransplant booster vaccination, the serum anti-HBs titers were positive pretransplant in all recipients with a median of 784 (8–18 736 IU/L); and the serum anti-HBc was positive in 8 (13%) recipients. The serum anti-HBs was positive in 60% of anti-HBc(−) donors after vaccination.

Forty (67%) recipients received postoperative vaccination. The reasons for not giving vaccination included the presence of preexisting high anti-HBs titers in 12 (60%) recipients and in recipients who require stronger immunusupression (8, 40%). The time to start vaccination posttransplant was 17 months (range, 4–42). The median vaccination episode was 3 (range, 1–19). The median titer before posttransplant vaccination was 23 IU/L (range, 0–461 IU/L), which increased to 1600 IU/L (range, 659–64 910 IU/L) after 1–3× of vaccination in 23 patients. Whereas in 17 patients, the median titer before posttransplant vaccination was 0 IU/L (range, 0–102 IU/L), which increased to 1600 IU/L (range, 141–16 000 IU/L) after >3× vaccinations. Only 1 patient responded poorly with titers rising from 102 to 141 IU/L. The sequential titers of anti-HBs were described in Table 2 based on the number of vaccination. Overall, 47 (78%) recipients achieved high-level titers (>1000 IU/L) of anti-HBs. The postvaccination results were described in detail according to grafts used (anti-HBc(−) vs. anti-HBc(+) donors) in Table 3. The median duration of lamivudine prophylaxis was 28 months (range, 20–42). The median follow-up periods were 51 and 57 months among recipients utilizing grafts from anti-HBc(−) and anti-HBc(+) donors, respectively. The median follow-up after discontinuing lamivudine prophylaxis was 31 months (range, 6–52) among recipients receiving anti-HBc(+) grafts. One (1) recipient (LDLT 107) who received an anti-HBc(−) graft died of pneumonia 18 months posttransplant. At the end of the follow-up period, two patients were negative for anti-HBs.

Table 2: The sequential titers of anti-HBs based on the number of posttransplant booster vaccination

Number of vaccination	No (n = 20)	≤3 times (n = 23)	>3 times (n = 17)
Prior to posttransplant vaccination (n, titer)			
<100	4 (20%), 11 (0–82)	18 (78%), 18 (0–54)	16 (94%), 5 (0–93)
>100, <1000	8 (40%), 310 (153–894)	5 (22%), 426 (128–461)	1 (6%), 102
>1000	8 (40%), 2657 (1142-32000)	0	0
Results after vaccination (n, titer)			
<100		0	0
>100, <1000		5 (22%), 900 (659–996)	4 (24%), 564 (141–742)
>1000		18 (78%) 3857 (1113–64 910)	13 (76%), 2500 (1125–16 000)
End of follow-up (n, titer)			
<100	6[1] (32%), 40 (0–96)	3[2] (13%), 42 (0–54)	2 (12%) , 43 (31–55)
>100, <1000	9 (47%), 482 (206–915)	8 (35%), 692 (153–960)	7 (41%), 595 (151–657)
>1000	4 (21%), 1600 (1145–2427)	12 (52%), 1600 (1063–7676)	8 (47%), 1400 (1056–9386)

The titers of anti-HBs were presented as median with range in IU/L.
n = number of patients.
One patient died in nonvaccinated group.
[1]One patient(LDLT 105) was negative for anti-HBs.
[2]One patient with *de novo* hepatitis B (LDLT 34). This recipient was negative for anti-HBs.

The titers of anti-HBs were >1000 IU/L in 24 patients and <1000 IU/L in 33.

Two (3.3%) recipients developed *de novo* HBV infection. One received a graft from an anti-HBc(+) donor; and another received a graft from an anti-HBc(−) donor. Both recipients were in the lower anti-HBs titer group (<1000 IU/L) as shown in Figure 1. They were also negative for anti-HBc preoperatively. The sequence analysis of *a* determinant of HBsAg (aa 116–160) did not show escape mutant although one silent point mutation was found compared to wild type (adr) as shown in Figure 2 (17). The incidence of *de novo* HBV was significantly higher in the lower titer group (15.4% vs. 0%, p = 0.04) as presented in Table 4. The first recipient (LDLT 52) received a graft from an anti-HBc(+) donor and had repeated vaccination, and recorded his highest anti-HBs titer (469 IU/L) at 30 months posttransplant. When lamivudine was discontinued, *de novo* HBV occurred 8 months later. Lamivudine was restarted; and HBsAg became negative again after 9 months of retreatment as shown in Figure 3. The other recipient (LDLT 34) received a graft from an anti-HBc(−) donor and achieved his highest anti-HBs titer (725 IU/L) at 15 months posttransplant with two vaccination episodes posttransplant. *De novo* HBV occurred on the 20th month posttransplant and lamivudine therapy was started. Adefovir dipivoxil was added in the management of this recipient because of a mutant strain that developed 5 years posttransplant.

Table 3: Results of vaccination based on donor anti-HBc status

Graft	Anti-HBc(−)	Anti-HBc(+)
Patient number	30	30
Patient with posttransplant vaccination	18	22
Time of starting vaccination (months)	17 (4–43)	14 (4–42)
Number of vaccination	3 (1–8)	3 (1–19)
Duration of lamivudine (months)	0	28 (20–42)
High titer (>1000 IU/L)	24 (80%)	23 (76.7%)
Follow-up after transplant (months)	51 (18–85)	57 (33–85)
Follow-up without lamivudine (months)	51 (18–85)	31 (6–52)

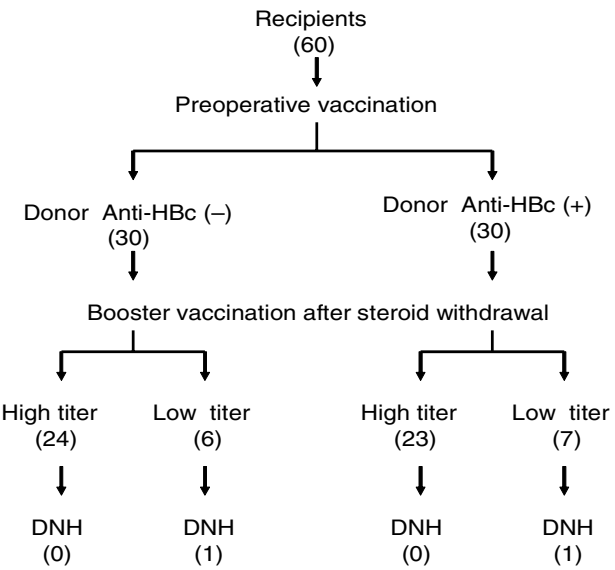

Figure 1: The number of patients based on donor anti-HBc status and outcomes of vaccination. High titer: anti-HBs > 1000 IU/L, Low titer: anti-HBs < 1000 IU/L, DNH: *de novo* HBV infection.

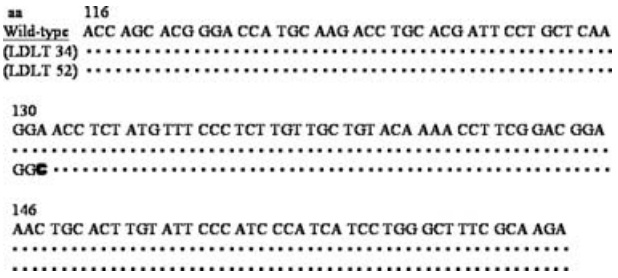

Figure 2: The nucleotide sequence of *a* determinant of HBsAg (aa 116–160) between wild type (adr) and two patients with *de novo* HBV infection. One silent point mutation occurred at aa 130(Gly) in LDLT 52. The mutated nucleotide is highlighted in bold font. aa: amino acid residue.

Discussion

Although the incidence of *de novo* HBV is lower among liver recipients from anti-HBc(−) donors than from anti-HBc(+) donors, there are other sources where HBV can be acquired. These sources include transfusion of blood and blood components, hospital personnel, etc. In our cohort, 8 (13%) children have been exposed to HBV since their pretransplant anti-HBc is positive. Several prophylactic regimens have been proposed to prevent *de novo* HBV in HBsAg(−) recipients who received grafts from anti-Hbc(+) donors. These include HBIG alone, HBIG + lamivudine, lamivudine alone and pretransplant vaccination + lamivudine (18). HBIG alone is not effective and 8% of recipients still develop *de novo* HBV (6). Further, its administration is costly and inconvenient, especially when given at high doses, and for long-term use. Lamivudine prophylaxis is certainly attractive because it is relatively less expensive, safe and convenient to administer. A major concern, however, with long-term use of lamivudine is the emergence of drug-resistant mutant strains. As monoprophylaxis for HBV recurrence, mutant strains have been reported to occur in 3.8–32% of recipients receiving long-term lamivudine (19).

Prophylaxis therapy using lamivudine or HBIG for recipients of liver coming from anti-HBc(−) donors is not justified because of high cost and potential side effects. Active immunization is a reasonable strategy to prevent *de novo* HBV in this subset of recipients. Only 2 (3.3%) recipients developed *de novo* HBV in our series of patients

Table 4: Incidence of *de novo* hepatitis B infection is lower in the higher titer group (15.4% vs. 0%, p = 0.04)

	De novo hepatitis B	
Anti-Hbs titer	Yes	No
(>1000 IU/L)	0	47
(<1000 IU/L)	2 (15.4%)	11

Fisher's exact test = 0.04.

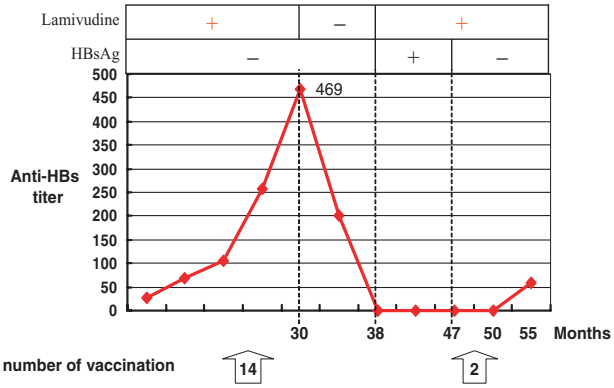

Figure 3: HBsAg and anti-HBs titers, and occurrence of *de novo* HBV. The patient had repeated vaccination and achieved the highest anti-HBs titer at 469 IU/L. When lamivudine was discontinued, *de novo* HBV occurred 8 months later. Lamivudine was restarted; and HBsAg became negative again after 9 months of retreatment.

who received either anti-HBc(−) or anti-HBc(+) grafts. Our findings show that vaccination is an effective method in preventing *de novo* HBV in HBV endemic areas because it is simple, cheap, universally accepted and is being given nationwide.

The protective anti-HBs titer against HBV infection is known to be 10 IU/L in the general population. However, there are no guidelines until now for patients receiving immunosuppressants after transplant. Some centers use HBIG to prevent *de novo* HBV and maintain the anti-HBs titer levels at >100 IU/L (8). Chang et al. reported that active immunization is effective in protecting young children

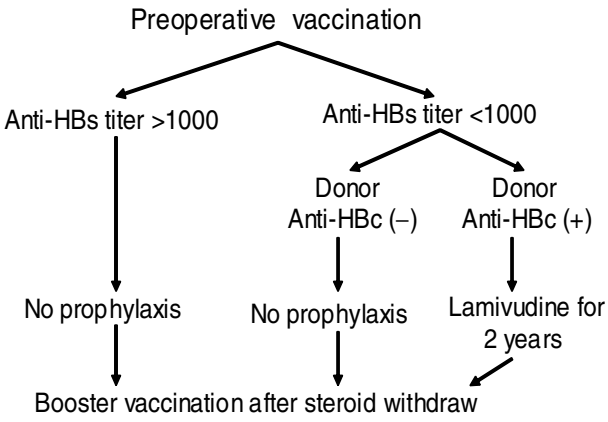

Figure 4: Algorithm for prophylaxis to prevent *de novo* HBV. For recipients with pretransplant anti-HBs titers >1000 IU/L or from anti-HBc(−) grafts, no prophylaxis is required. Recipients receiving anti-HBc(+) grafts require 2 years of lamivudine therapy if their anti-HBs titers are <1000 IU/L. Booster vaccination is given after steroid withdrawal.

receiving anti-HBc(+) grafts from *de novo* HBV by maintaining the anti-HBs titer levels at >20 IU/L and only one nonresponder developed *de novo* HBV in their series of 19 recipients. The median follow-up, however, was only 10 months; and it needs longer follow-up to validate the results because *de novo* HBV can occur beyond this time frame. The titers of anti-HBs wane off as time goes by. Following organ transplantation, recipients receiving lifelong immunosuppressants usually do not mount adequate immune responses as effectively as normal individuals. It is inevitable to boost immune responses by vaccination to achieve higher titers of antibody in these immunocompromised patients.

In our series, the incidence of *de novo* HBV was significantly higher in the lower titer group (15.4% vs. 0%, $p = 0.04$). *De novo* HBV recurrence occurred in recipients whose anti-HBs titers were >100 IU/L but <1000 IU/L. In the high-titer group (>1000 IU/L), none developed *de novo* HBV. Hence, we believe that simply maintaining anti-HBs titers at >100 IU/L is not enough for protection against developing *de novo* HBV.

Is it practical to maintain anti-HBs titer levels at the high range? It is generally accepted that the immune system is less capable of mounting effective immune responses among recipients receiving immunosupressants. T- and B-cell responses are impaired through blockade of cellular proliferation by calcineurin inhibitors and steroids after antigen stimulation as well as by inhibition of cytokine production (20). HBV vaccination for HBV-related cirrhosis to prevent HBV recurrence almost always fails. Although many transplant centers immunize transplant candidates to prevent *de novo* HBV using various methods, the response rates were still below 40% and some of the responders lost detectable anti-HBs posttransplant in the adult population (21,22). To optimize the response, the timing of vaccination appears to be critical. The first 6 months posttransplant appears to be a period where mounting immune responses are lowest since the recipients are usually on their highest doses of immunosupresssants. As a general rule, the primary immunization should be administrated pretransplant or when the dosages of immunosupresssants have been reduced posttransplant. In pediatric patients with cholestatic liver disease, vaccination will yield better immune response if given prior to transplant and results in a higher protection rate against *de novo* HBV posttransplant. In this series, our patients have acquired their vaccination at an earlier stage of their liver disease with booster doses while undergoing evaluation. Hence, all were anti-HBs(+) pretransplant. Routine HBV vaccination was given after steroid withdrawal or when recipients are at low-dose immunosuppression posttransplant. During early posttransplant period where the recipient receives high doses of immunosupression with low titer of anti-HBs, prophylactic lamivudine is administrated to high-risk recipients who received anti-HBc(+) grafts.

Another major concern for vaccination is the escape mutant of *a* determinant within HBsAg which has been raised after HBV vaccination and use of HBIG post liver transplantation (23,24). The patient develops *de novo* hepatitis B despite the presence of anti-HBs. Both our patients were fortunately negative for sequence analysis of *a* determinant. The incidence of escape mutant was below 4% in HBV-infected infants who received HBV vaccine or HBIG to an HBV-infected mother in Western countries and such mutations are only responsible for silent or occult HBV infection. In contrast, the incidence increased to 11–66% in liver transplantation recipients who experienced HBV reinfection after HBIG prophylaxis (25). Although the incidence of escape mutant when using HBV vaccine to prevent *de novo* HBV infection is uncertain, it would be lower compared to the regimen of HBIG, which are used in other centers. Thus, we believe that the benefits of HBV vaccination for prophylaxis of *de novo* hepatitis outweigh its potential side effects.

The median follow-up of the patients who received anti-HBc(+) grafts without lamivudine prophylaxis in this series was 37 months. This follow-up is longer than the median time (35 months; range, 14–39) for *de novo* HBV occurrence reported in our earlier study.

Based on our results, we recommend the following prophylaxis treatment to prevent *de novo* HBV in pediatric LDLT (Figure 4). For recipients with pretransplant anti-HBs titers >1000 IU/L, no prophylaxis is required but only a booster vaccination after steroid withdrawal. Likewise, recipients having titers <1000 IU/L and receiving anti-HBc(−) grafts require no prophylaxis and only a booster vaccination is given after steroid withdrawal. However, recipients receiving anti-HBc(+) grafts require 2 years of lamivudine therapy in addition to booster HBV vaccinations after steroid withdrawal.

In conclusion, *de novo* HBV is preventable. We have demonstrated that the use of active immunization and keeping anti-HBs titers >1000 IU/L are simple and effective methods of preventing *de novo* HBV among pediatric LDLT recipients.

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