ORIGINAL ARTICLE

Clinical Course of *De Novo* Hepatitis B Infection After Pediatric Liver Transplantation

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The characteristics of hepatitis B virus (HBV) in vaccinated children who acquire *de novo* HBV infections after orthotopic liver transplantation (OLT) remain largely unknown. The aim of this study was to explore HBV mutants in pediatric OLT recipients with *de novo* HBV infections. In all, 50 recipients underwent OLT between December 1997 and October 2005, and they were regularly checked for HBV serum markers from November 2005 to April 2009. Before OLT, all were hepatitis B surface antigen (HBsAg)–negative and under the coverage of the universal infant HBV vaccination program. Those who became HBsAg-positive after OLT were diagnosed with *de novo* HBV infection. HBV viral loads and full-length genome sequencing were determined when the diagnosis of *de novo* HBV infection was established. Nine patients (9/50, 18%) acquired *de novo* HBV infections after OLT. None had graft loss or fulminant hepatitis. Five cleared HBsAg, and 4 of the 5 even recovered with antibody to hepatitis B surface antigen (anti-HBs) formation. The other 4 were persistently HBsAg-positive. Mutations in the major S gene (681 base pairs) were discovered in 8 (88.9%) of the *de novo* HBV–infected children. Six of them harbored mutations within the "a" determinant region (codons 124-147), whereas the other 2 had mutations outside this region. These 2 cleared HBsAg and recovered with anti-HBs formation. HBV DNA levels were not different between those who cleared HBsAg and those who did not. In conclusion, surface mutants are frequent among pediatric liver transplant recipients with *de novo* HBV infections, but their clinical relevance requires further study. ***Liver Transpl 16:215-221, 2010.*** © **2010 AASLD.**

Received June 27, 2009; accepted October 11, 2009.

De novo hepatitis B virus (HBV) infection after orthotopic liver transplantation (OLT) has been recognized since early 1990.[1] It is defined as seroconversion from hepatitis B surface antigen (HBsAg)–negative status to HBsAg-positive status in recipients after organ transplantation.

Implementation of the universal HBV vaccination program in Taiwan has prevented perinatal transmission from carrier mothers to their babies.[2] Presently, pediatric OLT is indicated mostly for non–HBV-related liver diseases. However, the occurrence of *de novo* HBV infection in pediatric recipients receiving pre-OLT HBV vaccination is likely related to the endemic environment and especially the adoption of antibody to hepatitis B core antigen (anti-HBc)–positive but HBsAg-negative allografts.[3-6] An inadequate level of antibody to hepatitis B surface antigen (anti-HBs) is another risk factor.[3,6,7]

Pediatric data for *de novo* HBV infection after OLT are conflicting.[3,6,8] Some patients may become chronic HBsAg-positive carriers, whereas others may not. The liver injuries range from mild changes to

Abbreviations: ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBe, antibody to hepatitis B e antigen; anti-HBs, antibody to hepatitis B surface antigen; BA, biliary atresia; FK-506, tacrolimus; HAI, histology activity index; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IgM, immunoglobulin M; MMA, methylmalonic acidemia; ND, not determined; OLT, orthotopic liver transplantation; PCR, polymerase chain reaction; PSC, primary sclerosing cholangitis; UCD, urea cycle disorder; YMDD, tyrosine–methionine–aspartic acid–aspartic acid.

This study was supported by a grant (NSC 95-2314-B-002-283-MY3) from the National Science Council of Taiwan.

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DOI 10.1002/lt.21980
Published online in Wiley InterScience (www.interscience.wiley.com).

serious graft damage.[9-13] Moreover, HBV viral characteristics in this setting have never been clearly described. Because all of our pediatric OLT recipients were under vaccination coverage, we focused on HBV mutations that may be driven by vaccine pressure.

The HBV vaccine contains HBsAg protein, which includes the main antigenic determinant (the "a" determinant) of the surface gene region between amino acids 124 and 147 within the major hydrophilic loop.[14] The prevalence of "a" determinant mutations in HBV-positive children was reported to be about 20% after a nationwide HBV vaccination program in Taiwan.[15] For organ transplant recipients with HBV-related disease, the reported incidence of "a" determinant mutants in HBV reinfection after OLT has varied from 0% to 33%, and these immune-escape mutants might emerge under hepatitis B immune globulin (HBIG) immunoprophylaxis.[14,16-19]

In this study, we sought to characterize the incidence and clinical course of *de novo* HBV after OLT. Because the target subjects had pre-OLT HBV vaccination, their post-OLT *de novo* HBV infections were expected to undergo immune pressure similar to that in patients who receive HBIG prophylaxis to prevent HBV reinfection after OLT. We hypothesized that surface mutants, especially "a" determinant mutants, play a role in the development of *de novo* HBV infection in pediatric OLT recipients.

PATIENTS AND METHODS

Recipients

Fifty surviving patients (age < 18 years) who underwent OLT between December 1997 and October 2005 at the National Taiwan University Hospital were enrolled and were regularly followed until April 2009 (median follow-up: 6.7 years; range: 3.6-11.5 years). The underlying diseases indicating OLT were biliary atresia (n = 35), progressive familial intrahepatic cholestasis (n = 4), Alagille syndrome (n = 2), urea cycle disorder (n = 2), methylmalonic acidemia (n = 2), echovirus-related neonatal fulminant hepatitis (n = 1), primary sclerosing cholangitis (n = 1), hepatoblastoma (n = 1), Caroli disease (n = 1), and cryptogenic fulminant hepatitis (n = 1). All the subjects were seronegative for HBsAg and had received HBV vaccination prior to OLT as required by the universal neonatal vaccination program.[2] Among them, 33 (66%) received allografts from anti-HBc–positive and HBsAg-negative donors. Before OLT, 19 recipients (38%) were anti-HBc–positive, and 6 (12%) were negative for all HBV markers. Serum drug levels of immunosuppressants and liver function tests were monitored at least every month. Regular testing of post-OLT HBV serum markers, including HBsAg and anti-HBs titers and anti-HBc, was initiated in November 2005. Three of the 50 recipients (patients 1, 2, and 5) were already diagnosed with *de novo* HBV infection before the survey.

Donors

All donors were seronegative for HBsAg. If recipients developed *de novo* hepatitis B, serum samples of living donors were collected and tested for HBV DNA by polymerase chain reaction (PCR). A liver tissue sample was obtained from 1 donor who was seronegative for HBsAg but seropositive for anti-HBs and anti-HBc, and his HBV DNA sequence was compared with that of his recipient, who was infected with *de novo* HBV.

Serological and Virological Studies

Pre-OLT HBV serum markers, including HBsAg, anti-HBs, anti-HBc, and antibody to hepatitis C virus, were recorded for the donors and recipients. HBV serum markers were tested with enzyme immunoassays (Abbott Laboratories, North Chicago, IL). Alanine aminotransferase (ALT) and HBV serum markers, including HBsAg, titers of anti-HBs, anti-HBc, immunoglobulin M anti-HBc, and hepatitis B e antigen (HBeAg) and its antibody, were regularly checked in patients with *de novo* HBV infections. HBV viral loads and genotypes were checked in the patients' serum samples when the diagnosis of *de novo* HBV infection was established. The methods for HBV DNA quantification and the determination of HBV genotypes by real-time PCR have been described in detail previously.[20,21] Real-time PCR was performed with LightCycler analysis software (version 3.5, Roche Diagnostics Applied Science, Mannheim, Germany). The linear range of HBV DNA was 10^2 to 10^{11} copies/mL, and the sensitivity of the method was 5×10^2 copies/mL of HBV in serum.[21]

HBV Full-Length Sequences

The method of serum DNA extraction was described previously.[22] To amplify the full-length genome of the HBV strains, 3 overlapping subgenomic fragments covering the full length of the HBV genome were subjected to PCR. The modified primer pairs were fragment 1 forward (5′-TTTTTCACCTCTGCCTAATCATCT-3′, P1, nt 1821-1844), fragment 1 reverse (5′-AAAGA CAGGTACAGTAGAAG-3′, NP111038A, nt 2516-2497), fragment 2 forward 1 (5′-CATCTTCTTGTTGGTTCTT CTG-3′, NP210101A, nt 427-448), fragment 2 reverse (5′-AAAAAGTTGCATGGTGCTGGTG-3′, P2, nt 1825-1804), fragment 2 forward 2 (5′-GGAAACTCTATGT TTCCCTCATG-3′, NP2SF, nt 542-564), fragment 2 reverse (5′-AAAAAGTTGCATGGTGCTGGTG-3′, P2, nt 1825-1804), fragment 3 forward 1 (5′-ACTACTGTT GTTAGACGACG-3′, 11033C, nt 2336-2355), fragment 3 reverse 1 (5′-AATGGCACTAGTAAACTGAG-3′, NP410004C, nt 690-671), fragment 3 forward 2 (5′-GAGTGTGGATTCGCACTCCTCCAG-3′, NP3, nt 2268-2291), and fragment 3 reverse 2 (5′-GAGGACAA ACGGGCAACATACCTT-3′, NP4, nt 479-456).[22,23] The PCR conditions were those described by Liu et al.,[22] except that the extension time in each cycle was 120 s. The polymerase used was Combizyme DNA polymerase (InViTek GmbH, Berlin, Germany).

TABLE 1. Pre-OLT HBV Serological Status of *De Novo* HBV–Infected Recipients and Their Corresponding Donors

	Recipient					Donor
Number	Age for OLT (years)/Gender	Indication for OLT	Doses of HBV Vaccine Before OLT	Anti-HBs (mIU/mL) /Anti-HBc	Anti-HBs/ Anti-HBc	HBV DNA in Serum
1	6.5/female	BA	5	− (0.2)/−	+/+	−
2	0.7/male	MMA	2	+ (80.1)/+	+/+	−
3	0.9/female	BA	2	+ (>1000)/−	+/+	−
4	1.2/female	BA	3	+ (185.8)/−	−/−	−
5	9.7/male	BA	2	− (0.8)/+	+/+	−
6	13.1/female	PSC	4	+ (50.2)/−	−/+	−
7	0.9/male	BA	3	− (0.4)/−	−/+	−
8	17.4/female	BA	3	− (0.1)/+	+/+	ND*
9	3.3/female	UCD	4	+ (23.8)/−	+/+	−

*A serum sample was not available from the cadaveric donor.

The PCR products were isolated by electrophoresis, purified from the 1.5% agarose gel with the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Ltd., Taiwan), and then subjected to DNA sequencing with an automated ABI DNA sequencer (model 3730xl, Applied Biosystems, Foster City, CA) according to the manufacturer's instructions (ABI-Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits, version 3.1).

Determination of the Nucleotide and Amino Acid Sequences of the HBV Strains

Construction of the full-length HBV genome, alignments of paired nucleotide and amino acid sequences, and alignments of multiple nucleotide sequences were conducted with a network-based work station (http://workbench.sdsc.edu/CGI/BW.cgi).[24]

Antiviral Therapy Post-OLT

Lamivudine was administered to *de novo* HBV patients if the ALT level was above the normal limit (>40 U/L). Cellular rejection was excluded by histological examination before antiviral therapy was given. Doses of immunosuppressants (tacrolimus or cyclosporine) were maintained at a minimal level. Lamivudine was discontinued in patients in whom anti-HBs appeared and persisted (>6 months). Adefovir dipivoxil was used for patients with a tyrosine–methionine–aspartic acid–aspartic acid (YMDD) motif mutant breakthrough.

Histological Evaluation

Liver biopsy specimens for histological evaluation were fixed in formalin, embedded in paraffin for hematoxylin and eosin staining, and examined by the same pathologist. Disease activity was scored on the basis of the histology activity index.[25] Immunochemical staining of HBsAg and hepatitis B core antigen was conducted as described previously.[26]

Statistical Analysis

Fisher's exact test was used to examine the significance of any association between categorical data when the sample size was small. SPSS software was used for all statistical analyses (version 13.0 for Windows, SPSS, Chicago, IL), and a P value < 0.05 was deemed to be statistically significant.

RESULTS

De novo HBV infections were found in 9 of the 50 patients (18%) who underwent OLT. Eight underwent living donor liver transplantation, and 1 patient underwent cadaveric liver transplantation. The pre-OLT HBV serological status of the *de novo* HBV–infected recipients and the corresponding donors is listed in Table 1. Eight patients received allografts from anti-HBc–positive donors, and 6 of the donors were anti-HBs–positive. The transmission rate of *de novo* HBV infection was 24.2% (8/33) for recipients of anti-HBc–positive liver grafts. Donors with anti-HBc may influence the occurrence of *de novo* hepatitis B because of the inadequate protective levels of pre-OLT anti-HBs titers in recipients.[7] Three or more doses of pre-OLT HBV vaccine were given to 6 *de novo* HBV–infected children. Two of them were negative for all HBV serum markers, despite pre-OLT HBV vaccination. No HBV DNA was found in the donors' sera. All patients were negative for antibody to hepatitis C virus.

The clinical courses of the *de novo* hepatitis B patients after OLT were not uniform (Table 2 and Fig. 1). The peak ALT values were recorded as the highest levels during the whole follow-up period after the diagnosis of *de novo* HBV infection. No graft loss or fulminant hepatitis was found. For the 5 patients with HBsAg loss and undetectable HBV DNA levels during follow-up, 4 showed a resolving course with anti-HBs

TABLE 2. Virological, Serological, and Clinical Features of Patients with *De Novo* HBV Infection Post-OLT

Number	HAI Score	Peak ALT (U/L)[§]	Viral Load (log copies/mL)	HBeAg/ Anti-HBe[‖]	HBV Genotype	Surface Mutants	Post-OLT Survival (months)	HBsAg Clearance (within months)
1	10	528	6.1	+/−	B	E164G, F200Y, I213M	84	+ (3), R
2	ND	494	4.2	+/−	B	ND	71	+ (9.5), R
3	12	1026	4.1	+/−	B	P120S	43	+ (6.7), R
4	5	730	8.5	+/−	C	S113T, T126S,[‡] Q129H,[‡] T143S,[‡] K160R, Y161F, F200Y, I213L	47	+ (5), R
5	13	406	8.6	+/−	B	Q129H,[‡] L222F	138	+ (87)
6*	ND	124	5.6	+/−	B	G145R[‡]	81	−
7	6	320	7.3	+/−	B	T143M,[‡] F200Y, I213M	80	−
8[†]	ND	59	9.0	+/−	C	C85F, I110L, S113T, T126I,[‡] P127T,[‡] T143S,[‡] K160R, Y161F, T189I, F200Y, I213L	93	−
9*	ND	62	7.7	+/−	B	P142L,[‡] T143S,[‡] G145R[‡]	71	−

*The patient's HBV markers changed from HBsAg(+) and anti-HBs(−) to HBsAg(+) and anti-HBs(+).
[†]Patient 8 did not receive lamivudine treatment because her ALT value was transiently elevated over 40 U/L and returned to normal ranges during follow-up.
[‡]The locations of the "a" determinant were amino acids 124 to 147.
[§]The peak ALT values were recorded as the highest levels during the whole follow-up period after the diagnosis of *de novo* HBV infection.
[‖]The markers of HBeAg and anti-HBe were checked when the diagnosis of *de novo* HBV infection was established.

appearance (HBsAg seroconversion). For those with HBsAg persistence (n = 4) during follow-up of 3.0 to 3.6 years, HBeAg was persistently positive. The concomitant existence of HBsAg and anti-HBs was found in patients 6 and 9. Eight patients were treated with lamivudine. The YMDD motif mutant was detected in patient 5 after 18 months of lamivudine therapy, and he was then treated with adefovir dipivoxil instead of lamivudine. Five of those treated with antiviral agents showed HBsAg clearance at 3 to 87 months after diagnosis, and they also showed HBeAg clearance before the disappearance of HBsAg.

Five patients received tacrolimus at the time of *de novo* HBV infection; their serum levels ranged from 1 to 5 ng/mL. The remaining 4 patients received cyclosporine; their serum levels ranged from 78 to 159 ng/mL.

The histology activity index score was ≥10 in 3 *de novo* HBV–infected patients (patients 1, 3, and 5). Immunochemical staining for HBsAg was negative in all cases, but for hepatitis B core antigen, patient 7 showed positive nuclear staining and patient 1 showed positive cytoplasmic staining. Apart from viral hepatitis, patient 5 presented with cellular rejection.

The baseline HBV viral loads and genotypes were determined by real-time PCR once HBsAg was detected.

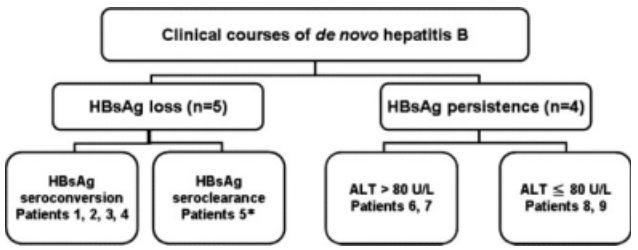

Figure 1. The 4 clinical courses of *de novo* hepatitis B included (1) HBsAg seroconversion (n = 4), (2) HBsAg clearance only (n = 1), (3) HBsAg persistence with ALT > 80 U/L (n = 2), and (4) HBsAg persistence with ALT < 80 U/L (n = 2). *Patients in this category had the YMDD motif mutation and were treated with adefovir dipivoxil. #The 4 patients with HBsAg persistence were all HBeAg-positive.

HBV DNA levels ranged from 10^4 to 10^8 copies/mL and from 10^5 to 10^9 copies/mL for patients with HBsAg loss (n = 5) and HBsAg persistence (n = 4), respectively (Table 2). When 10^5 copies/mL was adopted as the cut-off level to separate high viral loads from low viral loads,[27] the baseline HBV DNA levels were not different between the 2 groups ($P = 0.28$, Fisher's exact test). With respect to genotypes, 7 patients (77.8%) had genotype B, and the remaining 2 had genotype C; genotypes

```
Patient 1   ---------- ---------- ---------- ---------- --
Patient 3   -------s-- ---------- ---------- ---------- --
Patient 4   t--------- ---s--h--- ---------- s--------- --
Patient 5   ---------- ------h--- ---------- ---------- --
Patient 6   ---------- ---------- ---------- --r------- --
Patient 7   ---------- ---------- ---------- m--------- --
Patient 8   t--------- ---it----- ---------- s--------- --
Patient 9   ---------- ---------- ---------l s-r------- --

            113        123        133        143        153
adw         SSTTSTGPCK TCTTPAQGTS MFPSCCCTKP TDGNCTCIPI PS
```

Figure 2. Amino acid sequence of the "a" determinant of hepatitis B surface antigen (codons 124-147) in wild-type (adw) hepatitis B virus and 8 of the *de novo* hepatitis B virus–infected patients. The mutated amino acids are highlighted in lowercase.

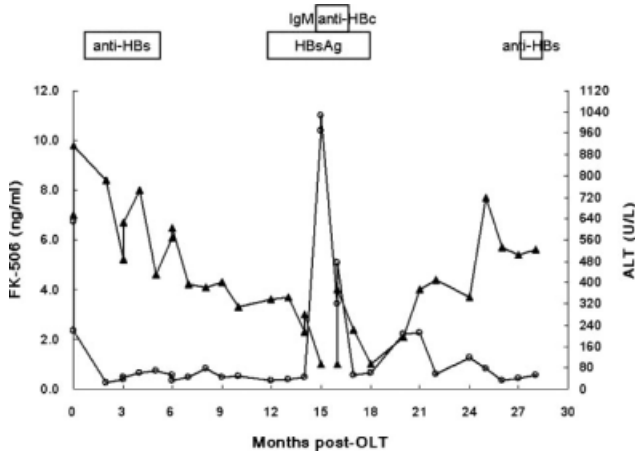

Figure 3. Temporal profiles of (▲) immunosuppressant levels, (○) ALT values, and HBV serum markers for patient 3, who experienced a successful resolving course of *de novo* HBV infection.

B and C were not related to HBsAg loss (4/7 versus 1/2, $P = 0.72$, Fisher's exact test).

The full-length HBV nucleotide sequence was determined in the serum samples of the patients, except for patient 2, whose viral load was too low to be sequenced. Mutations in the major S gene of HBV were discovered in all 8 *de novo* HBV–infected children (Table 2). Six of them harbored mutations within the "a" determinant region (at amino acids 126, 127, 129, 142, 143, and 145; Fig. 2). The other 2 (patients 1 and 3) had surface mutations outside the "a" determinant region and showed a resolving course of *de novo* HBV infection with anti-HBs formation. One patient (patient 4) harbored a basal core promoter mutation (A1762T/G1764A). None of the 8 patients acquired 1896 precore mutations with the diagnosis of *de novo* HBV infection.

To examine the possible source of *de novo* hepatitis B, a piece of stored liver tissue from patient 3's donor, obtained at harvest, was tested for HBV DNA. The donor, who was the recipient's father, was diagnosed with occult hepatitis B with the presence of HBV DNA in his liver tissue. The core regions of the HBV sequence in serum samples of patient 3 and her donor's stored liver tissue were further analyzed. The sequence homology in the region of nucleotides 1901 to 2452 was 99.6%. Despite the patient's *de novo* HBV infection, she underwent successful HBsAg seroconversion 9 months after HBsAg clearance (Fig. 3).

DISCUSSION

Adopting an anti-HBc–positive allograft is considered an important route of *de novo* HBV transmission.[3,4] Liver tissues of donors may harbor the HBV genome even though HBV serum markers indicate HBsAg clearance and anti-HBs seroconversion.[5,6] Although data on the infectivity of donors with occult hepatitis B are limited, it appears that HBsAg mutants may be the culprit.[16,28] Thus, OLT recipients who develop a *de novo* HBV mutant infection may have received it from occult HBV-infected donors with surface mutants in their grafts. According to this suggestion, such mutants and additional viral characteristics could probably also come from other potential sources of *de novo* HBV infection, including the transfusion of blood products, transmission by hospital personnel or close contacts, and reactivation of an occult HBV infection in the recipient.[10]

Eight (88.9%) of the 9 *de novo* HBV–infected children harbored surface mutants, and 6 (66.7%) had mutations within the "a" determinant region. The remaining patient might have shown the same mechanism, but the serum sample was unsuitable for sequencing. Surface mutants without mutations in the "a" determinant region were found in 2 patients who recovered with anti-HBs formation. The absence of "a" determinant mutants in *de novo* hepatitis B patients may signal a favorable prognosis.

Active and passive immunizations may not guarantee complete protection against surface mutants.[29] Such mutants have been documented in infants born to HBV-infected mothers after postnatal HBV vaccination and HBIG prophylaxis and in many recipients of liver transplants for HBV-related diseases who developed HBV reinfection despite HBIG prophylaxis.[16] The high percentage of HBsAg mutants in pediatric OLT recipients with *de novo* HBV infections may be related to pre-OLT HBV vaccination. HBsAg mutants emerge as an escape strategy in HBV-persistent infections in vaccine-failure children.[15,17-19,30]

Immunosuppression per se may not induce mutations but may lead to inadequate cellular and humoral immune responses to HBV infection.[17,19] In this study, those who did not recover with anti-HBs formation all contracted *de novo* hepatitis B with "a" determinant mutants. Compared with the wild-type HBV, the "a" determinant mutant is considered deficient in virion secretion and stability.[30] Despite this decreased fitness, such "a" determinant mutants seem to survive well in OLT recipients with persistent

de novo HBV infections. We suggest that immunosuppressed hosts are incapable of generating enough anti-HBs and restoring adequate cytotoxic T cell responses to neutralize and eliminate the immune-escape HBV "a" determinant mutants. However, when patients are tapered off their doses of immunosuppressants, host immunity can rebound, and they may recover from *de novo* HBV infections.

Several issues need to be considered with respect to the risk of *de novo* HBV infection, such as an anti-HBc–positive allograft, the protective titers of anti-HBs, the persistent use of immunosuppressants, and the possible reactivation of occult hepatitis B infections in organ recipients.[6,12,31] As our previous report showed,[7] we discovered that the pre-OLT anti-HBc status in recipients was not associated with the occurrence of *de novo* HBV infection ($P = 0.63$). The risk of *de novo* HBV infection resulted from either anti-HBc–positive liver grafts or the protective level of pre-OLT anti-HBs titers in recipients.

The clinical course of *de novo* HBV infection patients did not vary too much with respect to the recipients' pre-OLT serological status. Three *de novo* HBV–infected patients were anti-HBc–positive before OLT: 2 of them underwent HBsAg loss, and the other patient was persistently positive for HBsAg. However, they also received allografts from anti-HBc–positive donors. Because the allograft liver tissues were not available, we cannot completely rule out the possibility of HBV reactivation in recipients with positive anti-HBc before OLT. Four *de novo* HBV–infected patients were anti-HBs–positive alone: 2 of the 4 underwent HBsAg loss, and the other 2 were persistently positive for HBsAg. The remaining 2 patients were negative for all HBV markers before OLT and were supposed to be at higher risk for *de novo* HBV infection after OLT. One of the 2 experienced HBsAg clearance, and the other was still HBsAg-positive.

To prevent the occurrence of *de novo* HBV infection, all children on the waiting list for OLT should have protective anti-HBs titers by active immunization if possible.[6,7] Regular monitoring of anti-HBs titers and HBV serum markers in recipients post-OLT is essential.[3,9] Many authors also recommend the exclusion of anti-HBc–positive donors for liver transplantation. However, in areas hyperendemic for HBV, such as Taiwan, it is not feasible to exclude anti-HBc–positive donors currently.[32,33] Because of the universal HBV vaccination program, the HBsAg seroprevalence rate has declined from 10% to 0.6% in children in Taiwan.[2] The current anti-HBc seropositive rate is 3.7% in children in the general population.[2] In the next few decades, it will be feasible to select donors without previous HBV exposure and to minimize the risk of *de novo* hepatitis B transmission.

In addition to HBV vaccination, suggested prophylactic regimens for recipients at risk of *de novo* HBV infection include HBIG infusion during and after OLT and/or lamivudine.[3,5,8,9,34] In January 2006, we started a protocol for recipients receiving allografts from anti-HBc–positive donors. One intravenous dose of HBIG (100 IU/kg) in the anhepatic phase during the operation and lamivudine (3 mg/kg/day with a maximum of 100 mg/day) immediately after OLT are given. Lamivudine is administered for 19 months after OLT. If the anti-HBs antibody titer is good at that time, lamivudine is stopped. Otherwise, it is not discontinued until the patient responds to the booster HBV vaccines. We have regularly monitored post-OLT anti-HBs titers and HBV serum markers, and no new case of *de novo* HBV infection has been found to date. However, the 50 subjects enrolled in this study did not receive any prophylaxis except pre-OLT HBV vaccination. Without adequate prophylaxis, the incidence of *de novo* HBV infection in pediatric OLT recipients was high (18%). No consensus has been reached regarding the treatment of *de novo* hepatitis B after OLT.[6,10,35] The best treatment endpoints may be the clearance of HBsAg and appearance of anti-HBs. In this study, even after HBsAg loss, antiviral therapy was continued until an anti-HBs titer > 200 mIU/mL was achieved.

In conclusion, HBV surface mutants are frequent in pediatric OLT recipients with *de novo* HBV infections. Further studies are needed to establish causality and to determine whether specific mutations are responsible for the development of the disease. The clinical courses of *de novo* HBV infection are not ominous. In fact, over 50% of the patients showed successful HBsAg seroclearance with later anti-HBs appearance.

ACKNOWLEDGMENT

The authors thank Miss Hui-Chuan Lee for her work with the patient follow-up and data collection.

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