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ORIGINAL ARTICLE

# Liver transplantation for HBsAg-positive recipients using grafts from HBsAg-positive deceased donors

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**Keywords**
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## Summary

This study reports our experience using deceased donor liver grafts from HBsAg-positive donors. We performed eight cases of liver transplantation (LT) using grafts from deceased HBsAg-positive donors between November 2005 and October 2010. The median age of donors was 48 years (range: 26–64). HBV DNA in the serum of donors ranged from 44 to 395 IU/ml, but HBeAg in all donors was negative. Preoperative laboratory and liver biopsy samples revealed the absence of definitive cirrhotic features and hepatitis. All recipients showed HBsAg positive preoperatively except one patient with HBsAg(−) status post previous LT for HBV related liver cirrhosis. The median age was 60 years (range: 46–76) at LT. Post-LT antiviral management consisted of hepatitis B immunoglobulin and antiviral nucleos(t)ide analogues. The median follow-up period was 25.5 months (range: 14–82). Of eight recipients, two recipients experienced serum HBsAg and HBV DNA disappearance postoperatively. Three recipients died of HBV-unrelated causes. The remaining five recipients were stable with normal liver function and no marked pathologic changes on follow-up biopsies. This experience shows that LT using grafts from deceased HBsAg-positive donors is feasible, and may represent a valuable expansion of the pool of organ donors with appropriate antiviral management and monitoring.

## Introduction

Liver transplantation (LT) is an established therapeutic modality for patients with end-stage liver disease. The main limitation of this treatment is the shortage of organ donors. In Asia, the scarcity of deceased donors has resulted in living donor LT as a conventional procedure in LT and has also led to the increased use of marginal grafts.

In the Western Pacific region, 37 World Health Organization affiliated countries including China, Japan, South Korea, Philippines, and Vietnam account for nearly 50% of all chronic hepatitis B virus (HBV) infections worldwide [1]. A recent survey conducted in Korea reported the prevalence of HBV surface antigen (HBsAg) as high as 3.7% overall (male 4.4%, female 3.0%) [2]. This has had a significant adverse impact on organ donation from both living and deceased donors in this region. One study reported that 25–59% of living donors were positive for hepatitis B core antibody (HBcAb) [3]. Although *de novo* hepatitis can develop in recipients receiving a graft from an HBc antibody-positive donor, experience has shown that the infection may be prevented with prophylactic antiviral management and hepatitis B immunoglobulin. On the other hand, the use of HBsAg positive liver grafts has been contraindicated secondary to the high risk of recurrence of HBV leading to post-LT graft loss [4]. With advent of potent anti-viral agents and our extensive experience with management of HBV disease, we were encouraged to consider liver grafts from deceased HBsAg-positive donors with normal liver function and histology at donation.

Until now, only a few reports of limited cases exist describing the use of liver allografts from HBsAg-positive donors [5–8]. Therefore, the safety of HBsAg-positive liver grafts remains unknown. This study investigated the feasibility of using HBsAg-positive graft along with serial postoperative monitoring of recipient HBV status, liver enzymes, and liver biopsy for patients transplanted for-HBV-related disease.

## Materials and methods

We carried out 200 deceased donor LTs from November 2005 to July 2011 at Seoul National University Hospital. There were eight recipients who underwent LT using grafts from deceased HBsAg-positive donors and who had over 1 year of follow-up. Their median age was 60 years at time of LT (range: 46–76). Six patients were ABO identical and two were compatible cases.

Donors were selected by having no abnormal findings in the preoperative abdominal sonography and no cirrhotic features on the pathologic report on frozen section of liver tissue taken in donor operation. They did not have a history of significant hepatitis in the past (Table 1). Seven recipients were serum HBsAg positive and the other (recipient #2), who had been taking entecavir with HBIG, was HBsAg negative with HBsAb-positive status (>1000 IU/ml) after the first LT as a result of HBV-related hepatic failure. All subjects were informed of the high possibility of hepatitis B re-infection and resulting poor prognosis and provided informed consent.

### Clinical information about the donors and recipients

*Donors*

All donors (median age: 48 years; range: 26–64) were healthy carriers of HBsAg. The grafts from serum HBsAg-positive deceased donors showed no definite cirrhotic features and minimal or no inflammation. One donor had a history of lamivudine use. Human immunodeficiency virus, hepatitis A virus IgM, and anti-HCV were all negative. HBV DNA was detected in the sera from six donors (HBV DNA was not checked in the other two donors), but HBeAg was negative in all donors. CMV IgM, VDRL EBV IgM were all negative. HDV was not checked. Consistency of the grafts was soft and macro-vesicular fatty change was less than 15% in all grafts. After LT was performed, in two cases, frozen section was amended in the permanent pathology report as having partial fibrosis and septal fibrosis (Table 1).

*Recipients*

The median follow-up duration for recipients with HBV-related liver cirrhosis was 25.5 months (range: 14–82) after LT. Among them, three recipients had hepatocellular carcinoma and another patient had hepatocellular carcinoma with incidental cholangiocarcinoma that was found in explanted liver after LT. There was one case of combined liver and kidney transplantation as a result of hepatocellular carcinoma with ESRD and one re-transplantation case because of graft failure. Seven recipient's HDV was negative and CMV IgM, HIV, VDRL, EBV VCA IgM, EA IgM/IgG were all negative (Table 2).

### Prophylactic anti-HBV treatment

Hepatitis B immunoglobulin (HBIG; Green Cross Co., Seoul, Korea) was administered intravenously to all recipients with HBIG 10 000 or 20 000 units during the anhepatic period. HBIG 10 000 or 20 000 IU was given daily for 7 days postoperatively, then 10 000 or 20 000 IU weekly for the 4 weeks postoperative. As the patients were followed-up, HBIG infusion schedule was adjusted according to each patient profile [9]. For the patient whose HBsAg remained positive and sero-negative conversion was not achieved despite HBIG administration, HBIG was discontinued. As for the patients who did not take any antiviral medication before LT, we prescribed entecavir 0.5 mg a day postoperatively. Recurrence of HBV was defined as the re-appearance of HBsAg in the serum. The post-LT anti-HBs titer and clinical data were collected at every visit after surgery. Serum HBsAg, Anti-HBs, HBV DNA titer, and any breakthrough infection were routinely monitored in any patient with an abnormal liver function test and also in cases of a sudden decrease in the anti-HBs titer under HBIG therapy.

### Immunosuppression protocol

The immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetil (MMF), and steroid after induction with basiliximab. Steroids were tapered and discontinued within 8 months for all recipients.

### Lab examinations and pathology

To monitor serum HBV DNA titer, we used the COBAS AMPLICOR HBV monitor test (Roche Molecular Systems, Pleasanton, CA, USA), which has a lower detection limit of 20 copies/ml. HBV DNA titer was reported as copies/ml with a Roche Amplicor HBV monitor test before December 2006. Its values were converted to IU/ml (1 IU/ml = 5.82 copies/ml). Four mutations (rtL180M/V, rtV173L, rtM204V/I/S, and rtV207I) associated with lamivudine resistance and a single point mutation (G145R) associated with HBIG escape, three mutations (A181V, N236T and S202G/I) for adefovir, and 10 mutations (I169T, V173L,

**Table 1.** Clinical features of the donors.

| Donor | Sex | Age | Brain death causes | CPR time (min) | ICU stay (day) | Abdominal SONO | Infection | Liver biopsy | Medication before transplantation | HBV DNA titer (IU/ml) | HBsAg | Anti-HBs | HBcAb IgM/IgG | HBeAg /Ab | HAV IgM /Anti-HCV | Peak AST/ALT before TPL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 26 | Traumatic SAH SDH | 0 | 9 | Unremarkable | Pneumonia | Chronic hepatitis minimal lobular activity and porto-periportal activity, periportal fibrosis | None | Not done | 229.95 | – | –/+ | –/+ | –/– | 36/83 |
| 2 | M | 28 | Traumatic SAH ICH | 0 | 1 | Unremarkable | R/O pneumonia | Fatty change <5% portal inflammation | Lamivudine (May 2005–) | Weakly (+) | 210.08 | – | –/+ | –/+ | –/– | 46/72 |
| 3 | F | 64 | Left. MCA aneurysmal Rupture | 0 | 2 | Two hepatic cyst(12 cm, 3 cm) | No | Macrovesicular fatty change <5% | None | 44 | 5043 | – | –/+ | –/+ | –/– | 17/19 |
| 4 | M | 55 | Brain Tumor postop bleeding | 0 | 1 | No remarkable | No | Macrovesicular 15% | None | 128 | + | 0.3 | Not done | –/+ | –/– | 40/20 |
| 5 | M | 38 | Spantaneuous SAH, ICH | 0 | 1 | Hemangioma 1 cm S7 | No | Macrovesicular 10% | None | 395 | + | – | –/+ | –/+ | –/– | 139/49 |
| 6 | M | 52 | Seizure attack | 10 | 1 | Unremarkable | No | Macrovesicular <5% septal fibrosis with suspicious cirrhotic change, mild portal inflammation | None | 48 | + | – | –/+ | –/+ | –/– | 162/167 |
| 7 | F | 50 | Traumatic SAH, SDH | 0 | 1 | Unremarkable | No | Macrovesicular <5%, Chronic active hepaptitis with mild portal inflammation, septal fibrosis | None | 134 | + | – | –/+ | –/+ | –/– | 38/19 |
| 8 | F | 46 | Anaphylatic shock, Bee sting | 60 | 1 | R/O chronic liver disease | VRE recal | Macrovesicular <5% | None | Not done | >250 IU/ml | – | –/+ | –/+ | –/– | 84/77 |

AST, asparatate transaminase; ALT, alanine transaminase; CPR, cardiopumlonary resucitation; HBV, hepatitis B virus; HBsAg, HBV surface antigen; SAH, sub-arachnoid hemorrhage; ICH, intra-cerebral hemorrhage; SDH: sub-dural hemorrhage.

**Table 2.** Preoperative characteristics of the recipients.

| Recipient | Sex | Age at LT | Op date (D/M/Y) | Type of LT | Disease | Child | MELD | Preoperative management | Antiviral Mx. before LT (duration) | HBsAg (IU/ml) | HBsAb (IU/ml) | HBe Ag | HBe Ab | HBc Ab IgM/IgG | HDV | HBV DNA at LT | Peak HBV DNA | Drug-resistant mutation before transplantation | Drug-resistant mutation after TPL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #1 | M | 55 | 09/11/2005 | DDLT | HBV LC | B9 | 17 | | Lamivudine (1 month) | >250 | 0 | – | + | –/+ | – | 43800 | >200 000 | N.A.* | L180M M204V |
| #2 | M | 59 | 17/06/2007 | DDLT (Re-OP) | HBV, chronic rejection (1st DDLT August 2006) | C10 | 28 | | Entecavir | – | 287.3 | – | + | –/+ | – | Not detected | 563 000 | N.N.† | N.N. |
| #3 | M | 76 | 11/06/2009 | DDLT | HBV HCC (pT2Nx) Cholangio carcinoma (pT3) | C11 | 14 | TACE#5 PEIT#3 | Adefovir | >250 | 1.4 | – | – | –/+ | Not done | Not detected | >200 000 | N.A | N.A. |
| #4 | M | 61 | 10/09/2009 | DDLT with KT | HBV HCC ESRD (pT2) | B7 | 21 | TACE#13 PEIT#3 | None | >250 | 27.3 | + | – | –/+ | – | 14 700 000 | 42 700 000 | N.I.‡ | N.N. |
| #5 | M | 70 | 23/02/2010 | DDLT | HBV HCC (pT2N0) | C10 | 16 | TACE#9 PEIT#2 | None | 7.28 | 1.3 | – | + | –/+ | – | 142 | 142 | N.N. | N.N |
| #6 | F | 59 | 06/07/2010 | DDLT | HBV LC | C12 | 36 | | Adefovir Entecavir | 952.6 | Not done | – | + | –/+ | – | 243 | >110 000 000 | L180M, M204V Adefovir N236T | N.A. |
| #7 | F | 65 | 10/07/2010 | DDLT | HBV HCV HCC (totally necrotic nodules N0) | B7 | 17 | TACE#6 | Lamividine | 178.6 | 0 | – | + | –/+ | – | 331 | 331 | N.A. | N.A. |
| #8 | M | 46 | 15/07/2011 | DDLT | HBV LC | C12 | 32 | | Entecavir (2 month) | 3214.8 | 0 | – | + | –/+ | – | 772 | 8970 | N.I. | N.I. |

*DNA genotyping was not done.

†No mutation associated with drug-resistance was able to be detected because the serum HBV DNA titer was lower than 357 IU/ml.

‡No mutation associated with drug resistance was found using DNA genotyping.

DDLT, Deceased Donor Liver Transplantation; LT, liver transplantation; N.A., not available; N.N., not noticeable; N.I., not identifiable; HCC, hepatocellular carcinoma; HBV, hepatitis B virus, HDV, hepatitis D virus; ESRD, end-stage renal disease.

T184G/S/A/I/L, S202G, S202I, M250V) for entecavir were tested. Routine liver biopsy was performed on around postoperative day 7. Additional liver biopsy was done if acute rejection was highly suspicious or we needed to differentiate a disease from abnormal values of LFT. Immunohistochemical staining of HBsAg and HBcAg on the liver biopsy tissue was done. Post-LT monitoring included immunosuppressant trough level, anti-HBs, complete blood count, liver function panel, renal function panel, electrolyte, and coagulation panel at each visit. On occasion serum HBsAg and titer of HBV DNA were performed.

## Results

### Antiviral therapy before and after LT

Prior to LT, two recipients (#1 and #7) were medicated only with lamivudine, two recipients (#2 and #8) took entecavir only, and one recipient (#3) received only adefovir. Recipient #6 received a combination therapy consisting of adefovir and entecavir. Recipients #4 and #5 received no antiviral medications prior to LT (Table 2). Only entecavir was prescribed to recipients #3, #4, and #5 for HBV prophylaxis after LT. Adefovir was added to the treatment regimen of recipient #1 following hepatitis B activation with an YMDD variant and to the regimen of recipients #2 and #7 for HBV prophylaxis of HBV. Recipients #6 and #8 received the same antiviral agent prior and following LT (preoperative lamivudine was temporarily prescribed before the combination of entecavir and adefovir in recipient #6) (Table 3).

### Clinical outcomes after LT from deceased HBsAg-positive donors

Two of eight patients experienced HBsAg clearance during the median follow-up of 25.5 months (range: 14–82). During the HBV DNA assessment around postoperative day 7 (recipient #7 was postoperative 2 months), serum HBV DNA was positive in only two patients with very low levels (46 and 277 IU/ml, Table 3). At the last follow-up, serum HBV DNA was negative in eight recipients, with exception of recipient #1. In recipient #1, the HBV DNA titer increased sharply at 51 months postoperatively and YMDD mutation variants appeared. After addition of adefovir to the lamivudine regimen, liver function test and HBV DNA titer normalized. At present, recipient #1 continues to do well (Fig. 1).

Prior to re-transplantation of recipient #2 owing to graft failure from chronic rejection, HBsAg sero-negative conversion was achieved with hepatitis B immunoglobulin (HBIG) and entecavir, status post first deceased donor LT. Upon reception of HBsAg-positive graft by recipient #2, anti-HBs decreased sharply despite HBIG administration and resulted in HBsAg-positive status. Although HBV DNA was controlled by entecavir for postoperative 2 years, recipient #2 died of necrotizing pancreatitis following duodenal ulcer perforation at postoperative month 25 (Fig. 1).

Recipient #7 had a combined hepatitis B and hepatitis C infection. A biopsy performed at 1 year postoperatively revealed reactivation of viral hepatitis with septal fibrosis and mild lobular inflammation (Fig. 2). The postoperative HCV RNA titer increased to 14 890 989 IU/ml at postoperative month 2, while HBV DNA and HBsAg were undetectable in the serum. Therefore, the patient was started on a HCV therapeutic regimen consisting of pegylated interferon plus ribavirin (Fig. 1).

A total of three deaths were encountered in this study. Previously mentioned death occurred 25 months after LT because of necrotizing pancreatitis after duodenal ulcer perforation. The second death, patient #4 resulted from cholangiocarcinoma with multiple liver, lung, and peritoneal metastasis 23 months post operation. The explanted liver in this patient revealed incidental cholangiocarcinoma, which progressed post-LT. The third death occurred as a result of hepatocellular carcinoma with lung and bone metastasis. These mortalities were not directly related with hepatitis B. The remaining patients are stable and show normal liver function (Table 3).

### Evaluation of HBV infection in serial liver biopsy tissue after LT

Liver biopsy was performed in seven of eight patients on post-LT Day 7. Mild portal inflammation was observed in two patients. There was no inflammation by HBV infection in the lobular and portal areas in the remaining five patients (Fig. 2). Immunohistochemistry staining on postoperative day 7 biopsy in five patients revealed HBsAg-positive status; only recipient #6 was HBsAg negative.

One-year follow-up liver biopsy revealed absent HBsAg reactivity in both recipient #6 and #7 (Table 4). In recipient #1, moderate HBcAg staining and mild lobular inflammation was observed from a liver biopsy obtained 6 years after transplantation (Fig. 2). Most recently, the patient's serum HBV DNA level was 3 380 IU/ml and liver function test results were within normal ranges (total bilirubin 1.2 mg/dl, aspartate transaminase 16 IU/l, and alanine transaminase 12 IU/l). A 2-year follow-up liver biopsy in two patients showed moderate lobular activity in recipient #2 and no inflammation in recipient #5. Other two patients with HBsAg clearance revealed mild portal inflammation and recurrent viral hepatitis by HCV infection, respectively. In these patients, HBsAg and HBcAg were all negative at postoperative 1 year by immunohistochemistry staining (Table 4).

**Table 3.** Postoperative characteristics of the recipients.

| Recipient | F/U (month) | HBIG (Day 0–7, 2,3,4 Weeks) | Antiviral Mx. after LT | Drug resistant mutation after TPL | Result | Death causes | HBV recurrence | Postoperative first HBV DNA (IU/ml) | Peak HBV DNA (postoperative days, months) | Last HBV DNA | Anti HBs Peak level during F/U | Ishak grade and score [13] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Liver Biopsy POD 7–12 days | Last follow-up liver biopsy (month) |
| #1 | 82 | 20 000 (Day 0–7) 10 000 (2,3,4 weeks) | Lamivudine Adefovir | L180M M204V | Alive | | + | 46 | 58 100 000 (51 months) | 3380 | 392.7 | N0 C0 S2 P1 F0 | N2 C0 S0 P2 F1 (72) |
| #2 | 25 | 20 000 | Entecavir Adefovir | N.N. | Expire | Necrotizing pancreatitis | + | Not detected | 46 (23 months) | 46 | <10 | Not done | P1 C4 F3 P2 F1 (24) |
| #3 | 22 | 20 000 | Entecavir | N.A. | Expire portocaval LN meta (11/8/2009) | Disease progression | + | Not detected | 696 (9 months) | <20 | <9 | N0 C0 S0 P2 F0 | Not done |
| #4 | 22 | 10 000 | Entecavir | N.N. | Lung and bone metastasis (17/8/2010) | Disease progression | + | Not detected | <20 (11 months) | <20 | <32 | N0 C0 S0 P0 F1 | N0 C0 S1 P1 F1 (6) |
| #5 | 31 | 10 000 | Entecavir | N.A. | Alive | | + | <20 | Not detected | Not detected | <32 | N1 C0 S1 P1 F1 | N0 C0 S1 P1 F2 (24) |
| #6 | 27 | 20 000 | Entecavir Adefovir | N.A. | Alive | | – | <20 | Not detected | Not detected | >1000 | N0 C0 S0 P0 F0 | N0 C0 S0 P0 F1 (12) |
| #7 | 26 | 20 000 (Day 0–7,2 Weeks) 10 000 (3,4 Weeks) | Lamivudine Adefovir | N.A. | Alive | | – | Not detected / HCV 14 890 969 (2 months) | <20/HCV 7 815 622 (3 months) | Not detected | 547 | N1 C0 S3 P0 F2 | N2 C3 S3 P1 F4 (12) |
| #8 | 14 | 20 000 (Day 0–7) 1000 (2,3,4 Weeks) | Entecavir | N.A. | Alive | | + | <20 | 32 (1 month) | <20 | 4.1 | N0 C0 S2 P2 F0 | N0 C0 S2 P2 F1 (12) |

N, periportal necroinflammatory changes (0–4); C, confluent necrosis (0–6); S, focal (Spotty) necrosis, apoptosis and focal inflammation(0–4); P, portal inflammation (0–4); F, fibrosis (0–6); POD, post-operative days.

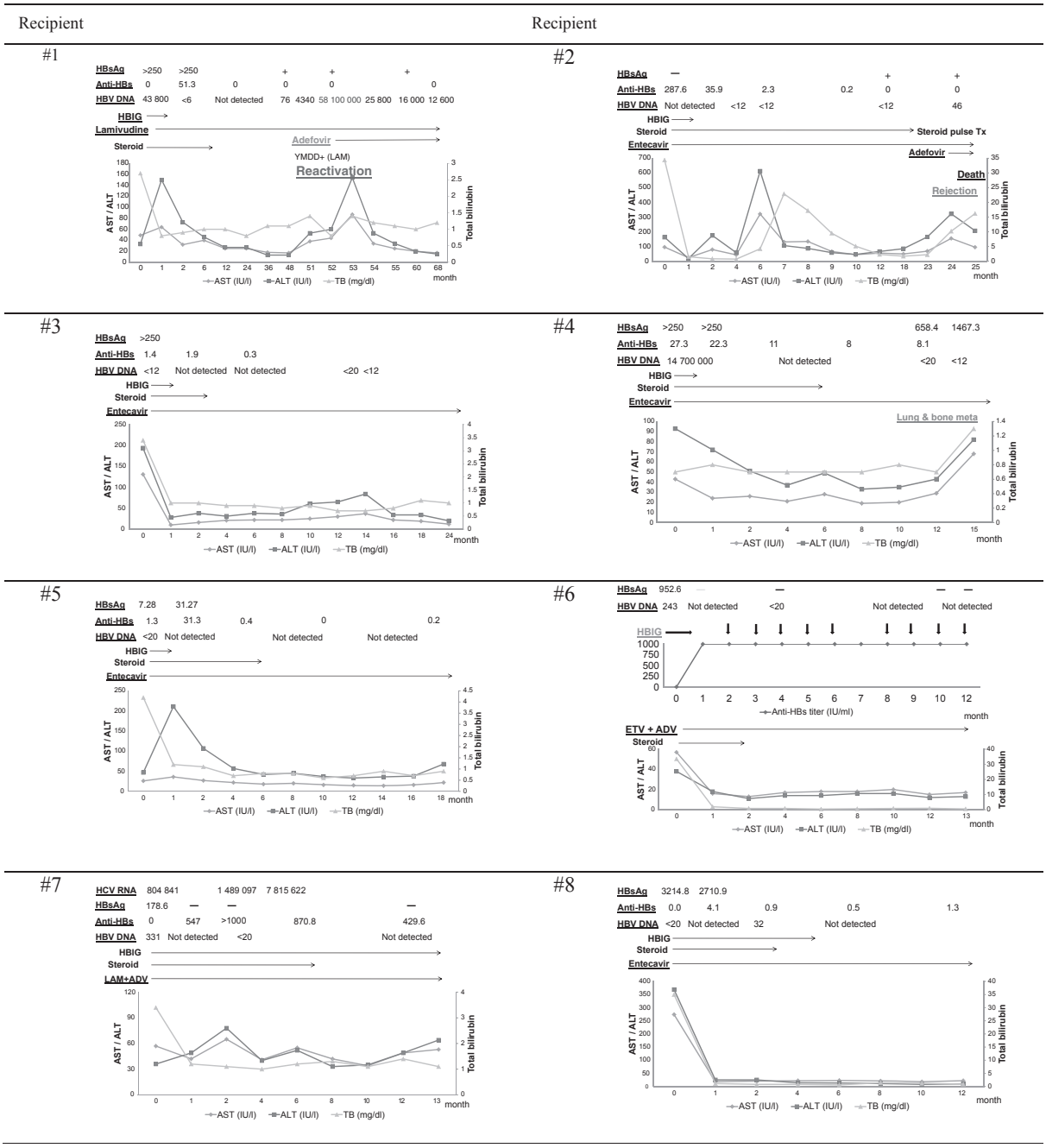
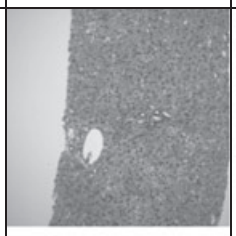
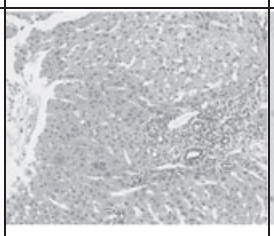
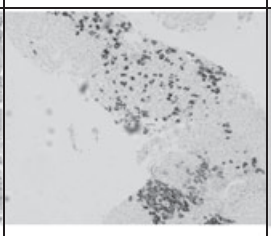
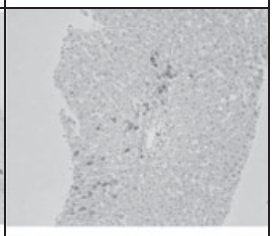
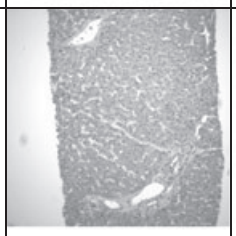
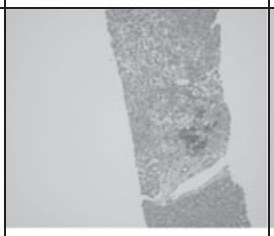
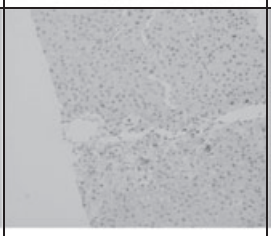
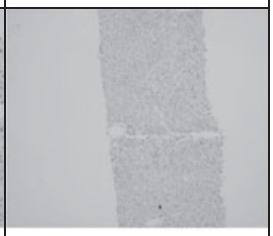
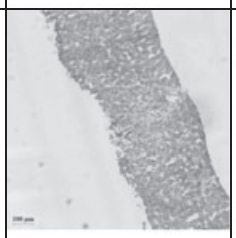
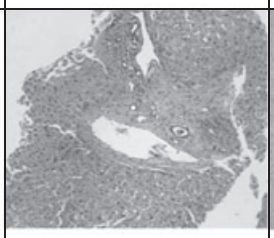
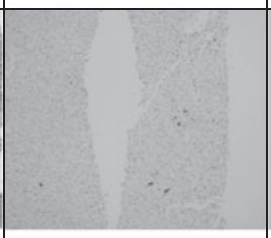
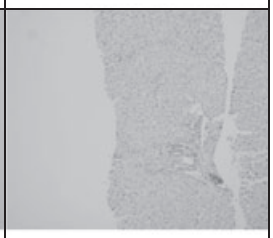
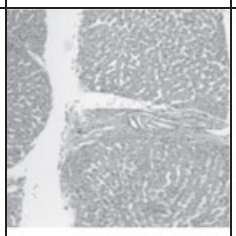
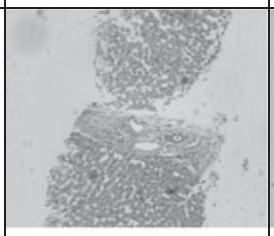
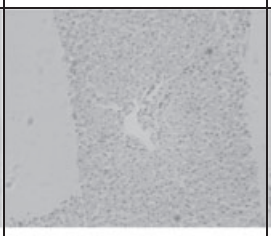
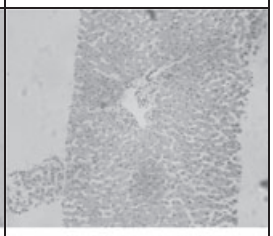
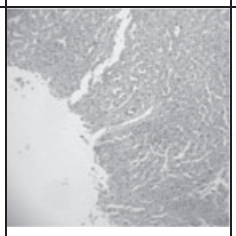
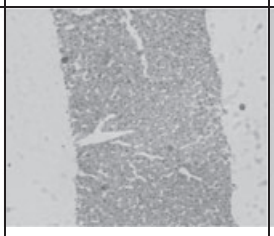
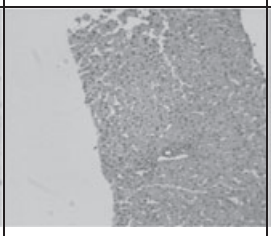
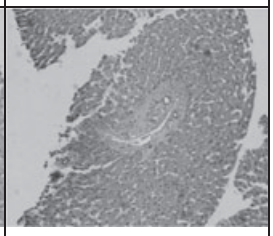



**Figure 1** Clinical course of recipients. Recipient #1: HBV DNA titer was well controlled by lamivudine and postoperative liver functions were normalized until 4 years after LT. At 51 months, liver function tests began to deteriorate, HBV DNA titer sharply increased, and YMDD variant appeared. Liver function tests began to normalize after adefovir was administered in conjunction with lamivudine, and HBV DNA titer has been steadily decreasing since then. Recipient #2: When the patient underwent re-transplantation, HBsAg sero-negative conversion had been succeeded by HBIG and entecavir after 1st DDLT. However, after the patient received HBsAg-positive grafts, HBs antibody titer sharply decreased despite HBIG administration and eventually HBsAg converted positive. Although, HBV DNA titer has been well controlled by entecavir continuously, the patient died as a result of chronic rejection at 25 months after LT. Recipient #6: HBsAg sero-negative conversion occurred via HBIG, entecavir and adefovir administration soon after LT, and the HBs antibody titer has been well maintained by HBIG administration thereafter. Moreover, the HBV DNA titer has been continuously undetectable. No adverse events have been observed up to now. Recipient #7: This patient had both HBV and HCV before LT. The patient achieved seroconversion by HBIG and lamivudine + adefovir administration, and HBV DNA has been undetectable until now. However, HCV was reactivated after LT; she is now on HCV treatment. Recipient #4 had lung and bone metastasis of HCC. He was lost to follow-up since November 25, 2010. Recipients #3, 5, 8 showed no specific clinical findings. HBsAg, HBV DNA and HCV RNA titers (IU/ml), Anti-HBs titers (mIU/ml).

| | Postoperative liver biopsy | | The last follow-up liver biopsy | | | |
|---|---|---|---|---|---|---|
| R | Post op | H/E staining | Post op | H/E staining | HBsAg stain | HBcAg stain |
| #1 | 7D |  | 6Y |  |  |  |
| | | x100, focal fibrosis | | x200, focal fibrosis | x100, positive | x100, positive |
| #2 | 2M |  | 2Y |  |  |  |
| | | x100 | | x40, ballooning change | x100, focal positive | x40, negative |
| #4 | 11D |  | 6M |  |  |  |
| | | x100 | | x100, focal periportal fibrosis | x40 focal positive | x40, negative |
| #5 | 9D |  | 20M |  |  |  |
| | | x100 | | x200, no fibrosis, no inflammation | x100, focal positive | X100, negative |
| #6 | 9D |  | 1Y |  |  |  |

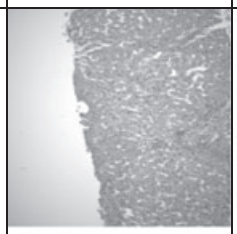
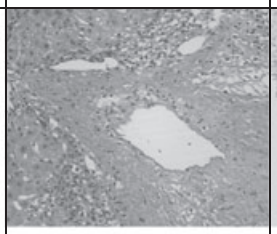
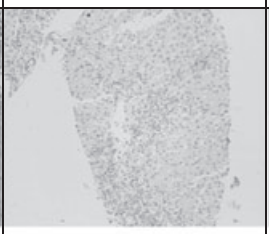
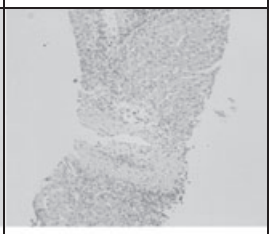
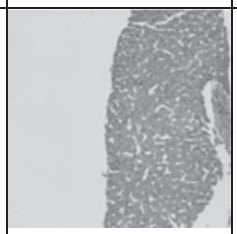
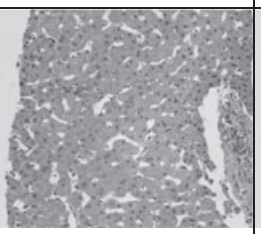
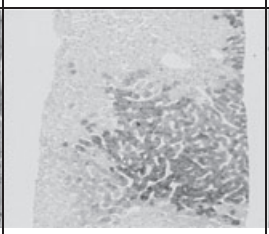
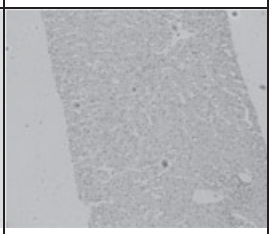
| | | | | | | |
|---|---|---|---|---|---|---|
| | | x100 | | x100, mild periportal inflammation | x100, negative | x100, negative |
| #7 | 10D |  | 1Y |  |  |  |
| | | X100, frequent spotty necrosis<br><br>Focal piecemeal necrosis | | x200, reactivated viral hepatitis, periportal inflammation | x100, negative | x100, negative |
| #8 | 7D |  | 1Y |  |  |  |
| | | x100, multifocal spotty necrosis, mild portal inflammation | | x200, mild portal inflammation, mild spotty necrosis | x100, positive | x100, negative |

**Figure 2** Continued.

## Discussion

The shortage of organ availability from deceased donors resulted in use of marginal donor organs. Liver grafts from HBsAg-positive donors have been precluded because of the high risk of HBV reactivation, with potential of graft loss. As a result of the limited clinical experience with HBsAg-positive donor grafts, most transplant centers do not use these grafts. However, our study clearly demonstrates the feasibility of a positive clinical outcome after LT with HBsAg grafts, despite most recipients (six of eight) being HBsAg-positive. Postoperative antiviral drugs effectively suppressed serum HBV DNA to undetectable levels in seven recipients. Although recipient #1 was HBsAg positive with the last HBV DNA titer 3380 IU/ml, the liver function tests were within normal limits without any evident signs of hepatitis following the addition of adefovir. Repeated liver biopsy with immunohistochemical staining for HBsAg and HBcAg, in conjunction with serum HBV DNA titer assessment, did not show any evidence of active hepatitis B in seven patients. These findings suggest that utilization of grafts from deceased HBsAg-positive donors may be feasible and that HBV can be controlled post transplantation by appropriate antiviral treatments.

Interestingly, two recipients had HBsAg-negative seroconversion after LT, comparable to LT from an HBsAg-negative donor. There were no definite differences in donor viral factors and HBIG schedules between the two

**Figure 2** Histologic changes of the HBsAg-positive grafts. Recipient #1: There was mild portal inflammation and the staining of both HBsAg and HBcAg was positive on postoperative 6 years liver biopsy. Recipient #2: Liver biopsy on postoperative 2 years showed that cholestasis with ballooning change in hepatocytes and staining of HBsAg was focal positive. Graft rejection was suspected clinically. Recipient #3: The histologic pictures are not shown because there was no his follow-up liver biopsy. Recipients #4, #5: There was no marked change on pathologic findings on follow-up liver biopsy. Recipient #6: There was mild portal inflammation and the staining of both HBsAg and HBcAg was negative on postoperative 1-year liver biopsy. Recipient #7: Masson-Trichrome staining showed septal fibrosis (not shown on this figure), mild lobular inflammation, but the staining of both HBsAg and HBcAg was negative on postoperative 1-year liver biopsy. HCV reactivation was diagnosed and she was on anti-HCV treatment. Recipient #8: At postoperative 1-year biopsy, mild periportal fibrosis, inflammation occurred and HBsAg stain was positive. Clinically these findings were suspicious for reactivated HBV infection.

**Table 4.** The result of HBsAg and HBcAg staining on recipient's postoperative liver biopsy.

| Recipient | POD 7 | POD 1 month | POD 6 months | POD 12 months | POD 24 months | POD 6 years |
|---|---|---|---|---|---|---|
| #1 | HBsAg: + | HBsAg: + | | | | HBsAg: + |
| | HBcAg: – | HBcAg: – | | | | HBcAg: + |
| #2 | Biopsy not done | | HBsAg: – | | HBsAg: + | |
| | | | | | HBcAg: – | |
| #3 | No IHC stain | | | | | |
| #4 | HBsAg: + | | HBsAg: + | | | |
| | HBcAg: – | | HBcAg: – | | | |
| #5 | HBsAg: + | | | | HBsAg: + | |
| | HBcAg: – | | | | HBcAg: – | |
| #6 | HBsAg: – | | | HBsAg: – | | |
| | HBcAg: – | | | HBcAg: – | | |
| #7 | HBsAg: + | | | HBsAg: – | | |
| | HBcAg: – | | | HBcAg: – | | |
| #8 | HBsAg: + | | | | | |
| | HBcAg: – | | | | | |

POD, Postoperative days.

sero-negative conversion patients and the remaining six patients. Of the eight recipients, seven had undetectable or very low HBV DNA level at the first post-LT assessment (within 7 days). Only two recipients with sero-negative conversion had anti-HBs titer over 100 IU during HBIG prophylaxis (within 1 month). Therefore, these two patients regularly received HBIG therapy in addition to oral antiviral drugs. Based on our results, HBsAg sero-negative conversion likely occurred during early postoperative period. We hypothesize that HBV may be effectively cleared by HBIG and HBV suppression may be achieved by oral antiviral drugs. A recent study [10] demonstrated the safety of grafts from HBsAg-positive donors in 10 patients with LT. In comparison to other recent studies, this study also demonstrates the safety of HBsAg-positive grafts by serial pathologic evaluations of graft tissue after LT. The definitive histologic diagnosis of recurrent hepatitis B is lobular and portal inflammation. Among the six patients with sustained HBsAg-positive status after LT with the exception of recipient #1, there was mild lobular and mild portal inflammation. However, we were unable to conclude that these mild inflammations in biopsy samples were associated with HBV recurrence, because these patients displayed normal liver function with undetectable or very low HBV DNA titer at the time of biopsy.

In this study, antiviral agent administered post-LT was entecavir in four, entecavir plus adefovir in two, and lamivudine plus adefovir in two recipients. Post-LT high potent antiviral therapy or appropriate anti-HBV medication based on drug mutations could have contributed to the effective control of HBV replication. Further studies are needed to make a definitive conclusion. HBIG administration for the first month may be helpful to eradicate circulating HBV particles during the early postoperative period. Unfortunately, we are unsure of the duration of HBIG use in LT from HBsAg-positive donor from the past clinical data. We used regular HBIG dose, similar to conventional HBV-related LT, during the first months after LT. If the anti-HBs titer was not sustained over 100 IU/ml for the first month**,** HBIG prophylaxis was withdrawn. It is established that high viral load or high HBsAg titer overrides the effect of HBIG as anti-HBs titer cannot reach the optimal titer of anti-HBs. In our experience, the time for withdrawal of HBIG would be 1–2 months after LT. Although our experience was limited to a small number of cases and the short follow-up duration, the strength of this study is the demonstration of serial liver biopsy after LT in this high-risk group. This study reveals minimal histologic changes of HBsAg-positive grafts, which have a high risk of HBV recurrence after LT, when treated with the appropriate HBV prophylaxis.

Previously, Loggi *et al.* [10] and Franchello *et al.* [8] reported that patients transplanted for HBV-related disease showed no clearance of HBsAg with lamivudine-based antiviral treatment. Despite the administration of HBIG, none achieved HBsAg sero-clearance after transplantation. In our study, two recipients who had HBV-related liver cirrhosis showed post-transplantation HBsAg clearance under dual medication of entecavir plus intravenous HBIG. This finding provides a new approaching to expand the door pool. With newly developed antiviral agents, the risk of HBV hepatitis recurrence can be reduced despite graft HBsAg positivity.

The limitations of our study were that patients' follow-up period was short and pathologic findings were cross-sectional results. We need to observe patients longitudinally with pathologic change in HBsAg-positive grafts under serial protocol biopsies. Also, we have to have the direct

attention to the occurrence of HCC because it is known that HBV infection leads to the development of HCC [11] and HBsAg positivity exhibited a stronger potential to promote tumor progression [12].

In conclusion, the utilization of grafts from deceased HBsAg-positive donors may be feasible and HBV can be controlled with graft stability if selection of grafts and postoperative antiviral treatment are appropriately managed. Long-term follow-up data and large-scale multi-center studies are required to confirm our findings.

## Authorship

YRC: performed research, analyzed data, wrote manuscript. JYC: interpretation of clinical data, revision of manuscript, approval of submitted manuscript. N-JY: designed and performed research, approval of submitted manuscript. KL: interpretation of pathologic data, participated in drafting the manuscript, approval of submitted manuscript. SM: acquisition of data, participated in drafting the manuscript, approval of submitted manuscript. HK, M-SP, S-WS and TY: performed research, participated in drafting the manuscript, approval of submitted manuscript. GH: collected data, analyzed data, approval of submitted manuscript. HWL: analyzed data, critical revision of manuscript, approval of submitted manuscript. K-WL: analyzed data, contributed important reagents, participated in drafting the manuscript, approval of submitted manuscript. K-SS: designed research, contributed important reagents, authored manuscript.

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