liver and that of the corresponding semi-automatically extracted liver=4.2(8.9) mL. Furthermore, various user-friendly features such as a procedural interface of virtual surgery planning were implemented into Dr. Liver for better usability.

Conclusions: It is concluded that Dr. Liver is a clinically effective tool to support liver surgery planning. More sophisticated features and functions are being developed and implemented to Dr. Liver to provide a surgeon with effective information for rational planning of liver surgery.

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Change of Hepatitis B Virus DNA and Covalently Closed Circular DNA Status in Liver Graft after Liver Transplantation

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Purpose: Despite effective prophylactic measures undertaken during liver transplantation for HBV related liver diseases, recurrence of HBV occurs at a rate of 5 ~10%. HBIG and antiviral agents are unable to eradicate the occult infection within the liver tissue as long as 10 years after LT even with successful prophylaxis. Covalently closed circular DNA (cccDNA), an intermediate in the life cycle of HBV, remains as a stable pool inside the hepatocyte, and may play a major role in limiting the success of prophylaxis. However there have been few reports concerning the change of HBV DNA and cccDNA after LT within the liver graft in respect to recurrence. The aim of this study is verify the validity a newly designed TaqManTM probe-based quantitative cccDNA and total HBV DNA PCR assay and to evaluate the change of HBV DNA within the liver graft after LT using this new assay.

Methods: PCR primers and probes were designed at a conserved sequence in aligning the reference standards of HBV genotypes A to E to measure total HBV DNA(tDNA) and primers and probes spanning across DR1 and DR2 were designed to detect cccDNA. To further increase the specificity of cccDNA amplification, samples were treated with Plasmid safeTM ATP-dependent DNase before amplification of cccDNA. The RNase P gene, a single-copy gene encoding the RNA moiety for the RNase P enzyme was used to detect and quantitate genomic copies of the hepatocytes. Plasmid pAM6 (ATCC, #45020) was inserted to HBV genome pBR322 and amplified for the standard reference curve to access absolute quantification of copies of HBV DNA's. Three liver samples from HBeAg positive and HBV DNA positive patients and one HBsAg negative sample were initially used to validate and standardize the real time PCR. Thereafter 150 samples from 70 patients who underwent LT from January 2006 to June 2007 were used. The biopsy#1 was taken before donor hepatectomy, #2 after reperfusion before closure of the recipient, and #3 (18 patients) 2 to 535 days after initial operation.

Results: HBV real-time PCR assay showed good linear range (tDNA Slope -3.566, R2 0.998; cccDNA Slope −3.487, R2 0.998), low percentage of coefficient of variation (CV%＜1.2), good dynamic range of at least 6 log10 (2.8×108 to 102 copies/reaction) and good efficiency (tDNA 89.0～93.3; cccDNA 93.4～95.6). tDNA was detected in 8% of anti-HBc positive donors. tDNA initially appears in 41.4% after reperfusion but gradually decreases to 22.2% but the cccDNA did not decrease (4.3% to 5.6%). Both tDNA and cccDNA in biopsy#2 was more frequently detected in preLT HBeAg(+) recipients (62.1 and 10% vs 26.8% and 0%, p=0.006 and p=0.067 respectively). PreLT recipient HBV DNA titer also had a strong correlation with tDNA and cccDNA in biopsy#2. Type of antiviral agents, duration of treatment and presence of mutation did not influence the presence of tDNA or cccDNA. Recurrence of HBV after LT was not related with donor anti-HBc serology, recipient HBeAg or HBV DNA titer. Also tDNA or cccDNA in donor liver and cccDNA in graft after reperfusion did not affect recurrence. However, tDNA had a strong correlation with recurrence.

Conclusion: Although tDNA may decrease with adequate prophylaxis, cccDNA does not and both are influenced by preLT viral replicative status. tDNA in liver tissue may serve as a strong surrogate marker of recurrence. pre LT viral clearance is necessary in order to keep HBV recurrence to a minimum.