# 学 位 論 文 の 要 旨

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学 位 論 文 名　QUANTITATIVE MEASURMENT OF HEPATITIS B VIRUS DNA IN DIFFERENT AREAS OF HEPATIC LOBULES IN PATIENTS WITH CHRONIC HEPATITIS B

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## 論 文 内 容 の 要 旨

### INTRODUCTION

The histopathology of chronic hepatitis B is characterized by chronic inflammatory infiltration, predominantly involving portal zones and extending into the hepatic lobules with piecemeal necrosis. However, the underlying cause of this distribution of cellular infiltration remains unknown. It has been considered that the targets of the immunoreaction in hepatic inflammation are HBV-related proteins expressed in the hepatocytes. There have been several reports concerning the distribution of HBV-related proteins and HBV-DNA observed directly using immunohistochemistry and in situ hybridization. However, these techniques are semi-quantitative and depend on visual identification of the stained cells. Therefore, to investigate the relative amounts of HBV-DNA in the portal and central areas of hepatic lobules, we measured HBV-DNA quantitatively in the peripheral and central zones of hepatic lobules using laser capture microdissection coupled with real-time quantitative polymerase chain reaction (PCR).

### MATERIALS AND METHODS

Fourteen patients with chronic hepatitis B admitted to Shimane University Hospital between 1998 and 2000 were studied. Serum samples and liver biopsy specimens were

collected. Serum alanine aminotransferase (ALT) level, HBV-DNA polymerase activity, hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were measured. The degrees of inflammation and fibrosis of the liver biopsy samples were assessed and graded according to the new Inuyama classification. The degree of necroinflammatory activity was graded from A0 to A3, and the degree of fibrosis from F0 to F4.

The sections were microdissected using a *LM200* laser capture microdissection system. Hepatocytes in the hepatic lobules were collected from the peripheral zone and from the central zone separately. After microdissection, real-time PCR for HBV-DNA was performed using DNA solution extracted from each sample. The results were normalized with corresponding samples of DNA whose concentration had been quantified by measuring the optical density at 260 nm.

Immunohistochemistry was performed for semi-quantitative detection of hepatitis B core antigen (HbcAg) in tissue sections of biopsy specimens. The presence and distribution of HBcAg-positive hepatocytes in the central intralobular and peripheral intralobular areas were evaluated separately. Statistical comparisons between two groups were made with the Mann-Whitney U test. Differences were considered significant at $P < 0.05$. Correlation analysis was performed using Spearman's rank correlation coefficient.

## RESULTS AND DISCUSSION

Patients without HBeAg showed a lower level of HBV-DNA polymerase in serum, but the difference was not significant. The amount of total intrahepatic HBV-DNA in patients positive for HBe antigen (n=10) was greater than that in HBeAg-negative patients (n=4, p=0.03). However, there was no correlation between serum ALT level and intrahepatic HBV-DNA.

When hepatocytes from peripheral zones and central zones of hepatic lobules were sampled separately, the amounts of HBV-DNA in the central and peripheral zones were not significantly different, either among all of the 14 patients ($10.1 \times 10^3$ vs $9.4 \times 10^3$ copies/$\mu$g DNA) or among the 10 HBeAg-positive patients($12.3 \times 10^3$ vs $11.5 \times 10^3$ copies/

$\mu$ g DNA) or 4 HBeAg-negative patients ($4.7 \times 10^3$ vs $4.3 \times 10^3$ copies／$\mu$ g DNA). Immunohistochemistry for HBcAg showed homogeneous staining in the cytoplasm of hepatocytes, and HBcAg-positive cells were evenly distributed in each hepatic lobule. These observations suggest that HBV-DNA and HBcAg are distributed homogeneously in hepatic lobules.

There was no correlation between histological findings and the amount of intrahepatic HBV-DNA. However, patients with high hepatitis activity tended to have lower HBV-DNA levels in the peripheral zone.

## CONCLUSION

HBV-DNA and HBcAg are distributed homogeneously in hepatic lobules.