ORIGINAL

HDV Infection Status in Tokushima Prefecture Based on Histopathological Evaluation : A Single-Institution Study

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Abstract : Hepatitis D virus (HDV) is a defective RNA virus requiring hepatitis B virus (HBV) for replication, and co-infection of them significantly accelerates liver inflammation, fibrosis, and hepatocarcinogenesis. Recent studies have reported that the prevalence of HDV infection is extremely high in certain regions, such as Mongolia, exceeding 50% among individuals infected with HBV. To clarify the potential presence of HDV infection in Japan, we retrospectively examined 95 surgically resected cases for HBV-related hepatocellular carcinoma (HCC) at Tokushima University Hospital between 2003 and 2023. Formalin-fixed, paraffin-embedded liver tissues were subjected to immunohistochemistry using an antibody against large and small delta antigens of HDV. Plasma samples from 12 patients were additionally tested by ELISA. As a result, none of the HBV-related HCC cases showed immunoreactivity for HDV antigens in either tumor or non-neoplastic tissues, and all available plasma samples were negative by ELISA. These findings suggest that HDV infection is either absent or extremely rare among patients with HBV-related HCC in this cohort. Our results align with prior seroprevalence estimates and provide histopathological evidence supporting minimal contribution of HDV to liver carcinogenesis in Japan, contrasting sharply with patterns in hyperendemic regions. J. Med. Invest. 73 : 52-54, February, 2026

Keywords **:** *hepatocellular carcinoma, immunostaining, hepatitis D virus, surgical dissection*

INTRODUCTION

Hepatitis D virus (HDV) is a unique defective RNA virus that relies on the envelope proteins of hepatitis B virus (HBV) for infection and replication, occurring in HBV-infected individuals either as a co-infection or as a superinfection. HDV infection not only exacerbates acute hepatitis but also accelerates the progression of inflammation and fibrosis in chronic hepatitis, thereby markedly increasing the risk of cirrhosis and hepatocellular carcinoma (HCC), as demonstrated by numerous epidemiological studies and meta-analyses (1-3). Moreover, HDV is not adequately suppressed by existing anti-HBV therapies, and persistent infection frequently results in difficult-to-treat chronic liver disease, underscoring its clinical significance.

In 2022, an estimated 258 million people were chronically infected with HBV worldwide ; although HDV affects only a subset, comprehensive global epidemiological data remain scarce (4). We previously investigated the epidemiology of HDV in Mongolia and reported that more than 50% of patients with HBV infection were co-infected with HDV (5). Such an exceptionally high prevalence is rare worldwide, and in this region HDV appears to be a major driver of hepatitis progression and hepatocarcinogenesis. In our analyses, HDV-positive patients had significantly higher fibrosis and inflammation scores, as well as a younger age at HCC onset, supporting the substantial impact of HDV on liver disease severity.

By contrast, the prevalence of HDV infection in Japan has been reported to be considerably lower. For instance, a recent study from Hokkaido University Hospital found anti-HDV antibodies in 10 of 601 HBV-infected patients (1.7%), with affected individuals showing more rapid progression of liver fibrosis (6). Global surveys adjusting for country-specific data have also estimated an anti-HDV seroprevalence of approximately 0.5% among HBsAg-positive individuals in Japan (4). However, most of these data are based on serological testing, and few studies have included direct evidence of HDV antigen expression or histopathological confirmation.

In addition, several meta-analyses have demonstrated that HDV infection significantly increases the risk of HCC compared with HBV monoinfection. Chang *et al*. analyzed 21 studies and reported an odds ratio of 2.08 (95% CI, 1.37–3.14) for HCC among patients with HBV/HDV co-infection (2). More recently, Bonacini *et al*. showed that HDV infection is associated with a higher risk of liver-related outcomes and overall mortality (7).

Despite these findings, studies examining HBV-related HCC cases in Japan using immunohistochemical detection of HDV antigens in liver tissue remain extremely limited. Consequently, the true frequency of HDV infection in this population—whether

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it is genuinely absent or present only in rare cases—remains unclear.

To clarify the potential presence of HDV infection in Japan, we retrospectively analyzed cases of HBV-related HCC diagnosed at Tokushima University Hospital, employing immunohistochemical methods to detect HDV antigens in liver tissue specimens. This study aims to provide new insights into the epidemiology of HDV in Japan, and even in the absence of positive cases, to furnish valuable evidence that the prevalence of HDV infection is either extremely low or negligible. Comparisons between high-endemic regions such as Mongolia and low-prevalence settings like Japan may also shed light on differences in transmission dynamics and environmental or behavioral risk factors for HDV.

PATIENTS AND METHODS

Patients and Samples

This retrospective study included 103 patients who underwent surgical resection for HBV–related liver cancer at Tokushima University Hospital, Japan, between January 2003 and December 2023. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks and routine histopathological diagnoses were available for all patients. Eight cases lacked preserved FFPE blocks ; therefore, the final analysis was conducted on the remaining 95 patients. Clinical data—including sex, age, serum biochemistry, and viral infection status—were retrieved from electronic medical records. The study was performed in accordance with the principles of the Declaration of Helsinki and its subsequent amendments, as well as comparable ethical standards, and was approved by the Ethical Review Board of Tokushima University Hospital (No. 4734).

Immunohistochemistry

Sections were prepared from both tumor tissue and corresponding non-neoplastic liver parenchyma (background liver) obtained from FFPE blocks. After deparaffinization, antigen retrieval was carried out by microwave heating for 12 min using ImmunoSaver solution, a 10% citraconic acid sodium with a pH of 6.9–7.8 (FUJIFILM Wako Pure Chemical, Osaka, Japan). A rabbit monoclonal antibody against the large and small delta antigens of HDV (clone HL1053, 1:800, Abcam, UK) was applied, followed by a horseradish peroxidase–conjugated anti-rabbit IgG secondary antibody (EnVision+ System, Agilent, USA). Color development was achieved with a diaminobenzidine substrate (Abcam). Staining was independently evaluated by experienced pathologists (H.O., K.K., and K.T.), with liver sections from HDV-positive Mongolian patients—identified in previous investigations—serving as positive controls.

ELISA

Stored plasma samples were available for 12 patients, in which circulating HDV antigen was assessed using a commercially available ELISA (Human Hepatitis D Virus IgG ELISA Kit ; Elabscience, China), according to the manufacturer's protocol.

RESULTS

Characteristics of patients are shown in Table 1. All 95 patients were serologically positive for HBsAg.

Immunohistochemical analysis was successfully performed on 95 evaluable cases of HBV-related liver cancer. In previously characterized positive controls—liver specimens from patients with confirmed HDV infection in Mongolia—distinct nuclear and cytoplasmic signals for HDV antigens were consistently observed in both tumor tissue and surrounding non-neoplastic parenchyma, validating the staining protocol (Figure 1A, B). By contrast, none of the 95 Japanese cases demonstrated immunoreactivity for HDV antigens in either neoplastic hepatocytes or the background liver parenchyma (Figure 1C, D). Staining was carefully reviewed by three independent pathologists, and all sections were deemed technically adequate, with clear preservation of tissue morphology and internal negative controls, suggesting that the absence of HDV signal was not attributable to fixation or procedural artifacts.

Because immunohistochemical studies are inherently limited by sampling error, we further examined residual plasma specimens when available. Stored sera or plasma from 12 patients were subjected to an enzyme-linked immunosorbent assay (ELISA) for circulating HDV antigen. In all samples, antigen levels were below the assay's lower limit of detection, providing no serological evidence of occult HDV infection. Taken together, these findings indicate that, within the studied cohort, HDV infection was either entirely absent or present only at levels below the sensitivity of current histological and serological methods.

Table 1. Characteristics of patients

	HBV-related HCC patients (n=95)
Age (yr), mean (range)	60.1 (36-87)
Male sex, n (%)	75 (78.9)
Serological markers	
Hepatitis B surface antigen, n (%)	95 (100.0)
Hepatitis B surface antibody, n (%)	10 (10.5)
Hepatitis B e antigen, n (%)	21 (22.1)
Hepatitis B e antibody, n (%)	64 (67.4)
Hepatitis B core antibody, n (%)	86 (90.5)
Hepatitis C virus antibody, n (%)	5 (5.3)

DISCUSSION

This study investigated the presence of HDV infection in patients with HBV-related HCC in Japan, using immunohistochemical analysis of liver tissue and serological assessment where plasma samples were available. In the present study, no evidence of HDV antigen was detected in the liver tissue of any of the 95 cases, and HDV antigen remained below the detection limit in all plasma specimens tested by ELISA. The lack of HDV positivity in this cohort contrasts sharply with data from countries with high endemicity, such as Mongolia, where we and others have reported co-infection rates exceeding 50-60% among HBV carriers (4, 5). Even within Japan, significant heterogeneity has been documented : in Miyako Island, a remote island of Okinawa Prefecture where HBV is most prevalent, antibody to HDV was present in 8.5% of HBsAg-positive individuals (8) ; a recent study from Hokkaido identified anti-HDV antibodies in 1.7% of HBV-infected patients (6), whereas expert generally agree on an anti-HDV prevalence of approximately 0.5% in Japan (4). Our results align with these population-based estimates. Taken together with the negative histopathological findings, these results indicate that HDV contributes little, if at all, to HBV-related HCC in Tokushima Prefecture.

The robustness of our methodology warrants consideration. Immunohistochemistry was optimized using liver tissue from

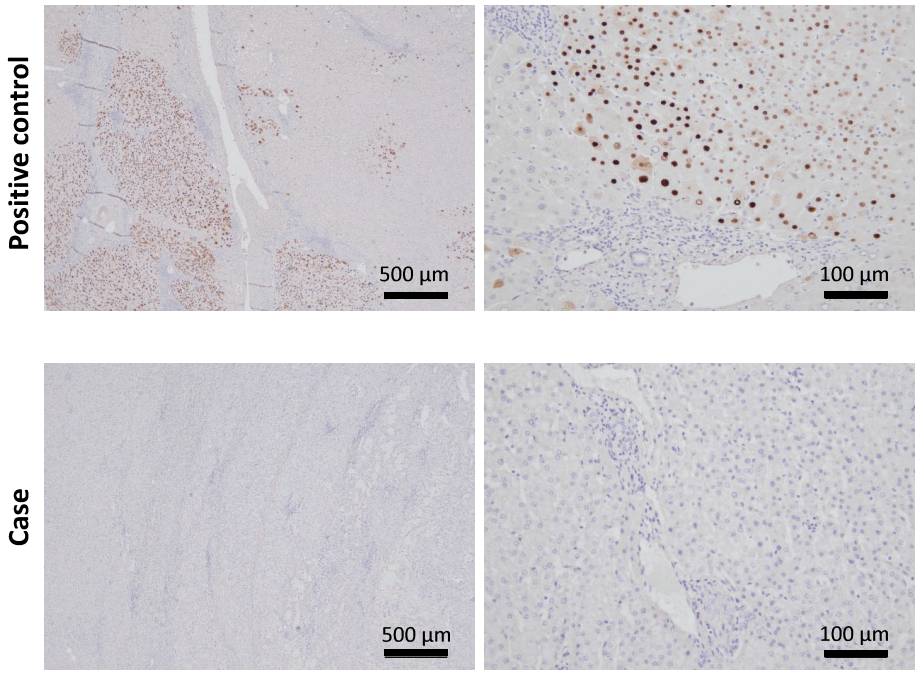

Figure 1. Immunohistochemical staining for hepatitis D virus (HDV) antigens in liver tissue from a positive control case (Mongolia, top panels). Abundant nuclear staining is observed in non-neoplastic (background) hepatocytes, with occasional positive cells also present within the tumor. In some areas, both nuclear and cytoplasmic immunoreactivity is evident. Representative sections from patients with HBV-related hepatocellular carcinoma in Tokushima (bottom panels). No HDV-positive cells were detected in either the tumor or adjacent liver parenchyma.

HDV-positive patients in Mongolia as external controls, ensuring adequate sensitivity and specificity of staining (5). All sections demonstrated good preservation of morphology and appropriate internal negative controls, indicating that the absence of HDV signal was not due to technical failure. Although sampling error cannot be entirely excluded—particularly given the patchy distribution of viral antigens—the consistent negativity across tumor and non-tumor tissues argues against significant occult infection. Furthermore, the supplementary ELISA analysis of stored plasma, although limited to a subset of patients, yielded concordant results, reinforcing the reliability of the findings.

Several limitations should be acknowledged. Although our series is larger than most prior histological investigations, it is still modest in size and represents a single-center experience. Plasma was available only for a minority of patients, and molecular assays such as HDV-RNA PCR were not performed. Future studies incorporating larger multicenter cohorts and adding sensitive molecular detection methods would be valuable to clarify whether subclinical or focal HDV infection exists across Japan.

COMPETING INTERESTS

All authors declare no conflict of interest for this article.

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