Replication and infectivity of hepatitis B virus in HBV-related glomerulonephritis

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Received 31 January 2008; received in revised form 29 July 2008; accepted 6 August 2008

Corresponding Editor: Jane Zuckerman, London, UK

http://intl.elsevierhealth.com/journals/ijid

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doi:10.1016/j.ijid.2008.08.014

Keywords
Hepatitis B virus-related glomerulonephritis;
Renal tissue;
HBsAg;
HBcAg;
HBeAg;
cccDNA

Summary
Objective: To examine the presence of hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) in renal tissues from patients with HBV-related glomerulonephritis.
Methods: Renal tissue biopsies taken from patients with HBV-related glomerulonephritis and two control groups were prepared for immunocytochemical detection of HBsAg and HBcAg. HBV cccDNA was examined using a nested PCR.
Results: Of the 63 HBV-related glomerulonephritis patients studied, HBsAg was present in the renal tissues of 48 (76.2%) and HBcAg in the renal tissues of 27 (42.9%). The HBsAg and HBcAg positive rates in HBV-related glomerulonephritis patients were higher than those of the 20 patients with non-HBV-related glomerulonephritis (p < 0.05). However, there was no significant difference when the HBV-related glomerulonephritis patients were compared with 12 patients with renal tuberculosis, renal atrophy, renal calculus, and renal tumor with positive serum HBV markers. In patients with HBV-related glomerulonephritis, there was no significant difference in HBsAg and HBcAg positive rates in renal tissue between patients with and without serum hepatitis B e antigen (HBeAg). By nested PCR, two of five patients with HBV-related glomerulonephritis were positive for HBV cccDNA.
**Introduction**

Hepatitis B infection is common in the Chinese population. Hepatitis B surface antigen (HBsAg) is found in the serum of about 10% in China. HSbsAg and hepatitis B core antigen (HBcAg) in renal tissues were detected by immunocytochemistry. The reagents used were purchased from Zhongshan Biological Limited Company, Beijing, China.

**Materials and methods**

**Patients**

The study population comprised 63 inpatients with HBV-related glomerulonephritis (55 males and eight females, with ages ranging from 15 to 50 years), who had attended the Renal Clinic of Xiangya Hospital, Central South University, between April 1995 and April 2003. Twenty non-HBV-related glomerulonephritis patients (17 males and threes female, with ages ranging from 17 to 55 years) and 12 patients with renal tuberculosis, renal atrophy, renal calculus, and renal tuberculosis recruited between March and April 2003. The diagnosis of HBV-related glomerulonephritis was based on established criteria:1 patients with serum and renal tissue HBV antigens with symptoms and signs of glomerulonephritis and without other secondary diseases such as lupus nephritis were diagnosed as HBV-related glomerulonephritis. ELISA was used to detect serum HBV markers. Renal tissues were processed and stained by hematoxylin–eosin staining, periodic acid–Schiff reaction, periodic acid–methenamine silver staining method, and Masson staining. Pathologic features of the renal tissues were examined by light microscopy. HBsAg and hepatitis B core antigen (HBcAg) in renal tissues were detected by immunocytochemistry. The reagents used were purchased from Zhongshan Biological Limited Company, Beijing, China.

**Detection of HBV cccDNA by nested PCR**

External and internal primers were designed according to the genomic X to C region and X to pre-C region of HBV, respectively, both of which spanned the two nicks DR1 and DR2. Primer sequences were as follows: external primer, upstream sequence 5′-CGACCAAGGCCCACCCTCTC-3′; downstream sequence 5′-GGGAAGACTCAAGGCAAA-3′; the length of PCR product was 462 bp. Internal primer, upstream sequence 5′-ACTCCCCGTGTGCTTTCTCATC-3′; downstream sequence 5′-AGCTTGGAGCCTTTGAACAGTA-3′; the length of PCR product was 335 bp.

Renal tissues were lysed with sodium dodecyl sulfate (SDS), and DNA was isolated by phenol and chloroform. Thirty cycles of DNA amplification were carried out in a total volume of 30 µL using external primer. The condition of each cycle was: denaturing at 94 °C for 5 min, then 94 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final extension of 72 °C for 10 min. A nested PCR was performed using internal primer. The reaction mixture was cycled in the same conditions. The PCR products were separated by electrophoresis at 80 V for 50 min in a 2.5% agarose gel, 1× TBE buffer, and stained with ethidium bromide. The PCR products were visualized in a UV-transilluminator and the images photographed with a digital camera to detect the expected 335 bp strap. Renal tissues from patients with non-HBV-related glomerulonephritis and hepatic tissues from patients with chronic HBV infection were used as negative and positive controls, respectively. DNA marker, Taq DNA polymerase, and dNTP were purchased from the TaKaRa Company, Dalian, China. The primer was synthesized by Bioasia Company, Shanghai, China.

All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

**Results**

All 63 patients with HBV-related glomerulonephritis had HBsAg in their serum, and 50 of these patients were also hepatitis B e antigen (HBeAg) positive. Of the 63 patients, 42 (66.7%) had membranous nephropathy and 21 (33.3%) had mesangiproliferative glomerulonephritis. There was obvious and irregular thickening of the glomeruli capillaries in membranous nephropathy, and the diameter of the capillaries was decreased; however, thickening in the mesangial cells, endothelial cells, and epithelial cells was not apparent. The thickened basal membrane was chain-shaped. Various
sizes of immune complex were deposited in the epithelial cells, glomerular mesangium, basal membrane, and endothelial cells. In cases of mesangioproliferative glomerulonephritis, moderate thickening was observed in the glomerular mesangium, but the glomerulus capillaries were normal. Clusters of immune complex were found deposited in the glomerular mesangium.

The results of immunocytochemistry indicated that HBsAg and HBCag were present in renal tissues, as shown in Figures 1 and 2. The positive rates of HBsAg and HBCag in renal tissue were 76.2% (48/63) and 42.9% (27/63), respectively. HBsAg and HBCag were predominantly within the kidney cells (glomerular mesangium and endothelial cells) and vascular endothelial cells, depositing linearly or in clusters.

According to the HBV markers in serum, 63 HBV-related glomerulonephritis patients were categorized into two groups: patients with and without HBeAg in the serum. The positive rates of HBsAg and HBCag in renal tissues of these two groups showed no significant difference (Table 1).

HBsAg and HBCag were not detected in the renal tissues of 20 non-HBV-related glomerulonephritis patients (Table 2). The renal tissue HBsAg and HBCag expression rates were 75% (8/12) in the twelve other renal patients with HBV markers (Table 3).

Of the five HBV-related glomerulonephritis patients tested, HBV cccDNA was detected in the renal tissues of two (Figure 3).

Discussion

HBV is not a virus that is found only in liver cells. Animal experiments have confirmed that it can exist and replicate in various other organs and tissues. Wang et al., carrying out research on HBV perinatal transmission, found that HBV could be located outside the liver in many organs or tissues in the fetus.12

Because of the high incidence of HBV infection, HBV-related glomerulonephritis is a well-known clinical disease and is an important type of secondary glomerulonephritis in China.13 Compared with those areas with low HBV infection rates, such as Western Europe and North America, it is noticeable that HBV-related glomerulonephritis accounts for a larger proportion of nephritis in China.

HBsAg and HBCag immune complexes from the circulation can be deposited in the glomerular mesangium and endothelial cells, leading to mesangioproliferative glomerulonephritis or other nephritis.14 The results of this study indicate that both HBsAg and HBCag are present in the glomerular mesangium and basal membrane in HBV-related glomerulonephritis. We found that the renal tissue HBsAg and HBCag positive rates in HBV-related glomerulonephritis are higher than those in non-HBV-related glomerulonephritis patients, but not higher than in other renal patients with HBV markers. We also found no significant difference when comparing the renal tissue positive rates of HBsAg and HBCag between

Figure 1  HBsAg was detected in renal tissue by immunocytochemistry. (A) HBsAg positive; (B) HBsAg negative.

Figure 2  HBCag was detected in renal tissue by immunocytochemistry. (A) HBCag positive; (B) HBCag negative.
HBV-related glomerulonephritis patients with and without serum HBeAg.

HBV is a partially double-stranded DNA virus of the Hepadnaviridae family. The length of the HBV genome is 3.2 kb. There are several types of HBV DNA according to the stage of replication, such as cccDNA, relaxed circular DNA (rcDNA), single-stranded DNA, and integrated DNA. In the lifecycle of HBV, cccDNA is a template for transcription of viral pregenomic RNA and all messenger RNA, which play an important role in HBV replication and the establishment of HBV infection.

Because of the existence of several types of HBV DNA at the different replication stages, it is rather difficult to detect cccDNA. In this study, HBV cccDNA was examined in renal tissue biopsies from patients with HBV-related glomerulonephritis using a nested PCR. The two sets of primers spanning the two nicks were well designed so that rcDNA would not be amplified, but cccDNA would specifically be amplified because of the two strands.

HBV can cause pathologic changes in the kidney in four ways. First, the HBV antigens in the circulation can be deposited in the kidney and lead to renal immune lesions. Second, HBV antigens synthesized in situ due to direct HBV infection of the kidney can result in immune complex nephropathy or cell immunoreaction nephropathy (cytopathic effect of HBV). Third, HBV-related glomerulonephritis may be a kind of renal lesion caused by HBV replication through various mechanisms. Finally, HBV-related glomerulonephritis may not be an independent disease but one of the important extrahepatic manifestations, which results from immune

Table 1 HBsAg and HBcAg expression in renal tissues of 63 patients with HBV-related glomerulonephritis.

<table>
<thead>
<tr>
<th></th>
<th>HBsAg in renal tissue</th>
<th>HBcAg in renal tissue</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Patients with negative HBeAg</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Patients with positive HBeAg</td>
<td>37</td>
<td>13</td>
</tr>
</tbody>
</table>

HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen.

a Chi-square = 0.189; p = 0.663.

b Chi-square = 0.129; p = 0.719.

Table 2 Comparison of renal tissue expression of HBV markers between HBV-related glomerulonephritis and non-HBV-related glomerulonephritis patients.

<table>
<thead>
<tr>
<th></th>
<th>HBsAg in renal tissue</th>
<th>HBcAg in renal tissue</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>HBV-related glomerulonephritis</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>Non-HBV-related glomerulonephritis</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen.

a Chi-square = 36.14; p = 0.00.

b Chi-square = 12.7; p = 0.00.

Table 3 Comparison of renal tissue expression of HBV markers between HBV-related glomerulonephritis patients and other renal patients.

<table>
<thead>
<tr>
<th></th>
<th>HBsAg in renal tissue</th>
<th>HBcAg in renal tissue</th>
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<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>HBV-related glomerulonephritis</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>Other renal patients with positive HBV markers</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen.

a Chi-square = 0.111; p = 0.739.

b Chi-square = 2.30; p = 0.13.

Figure 3 Detection of HBV cccDNA in renal tissues by nested PCR. M: DNA marker; lane 1: negative control; lane 2: positive control; lanes 5 and 7: positive samples.
failure in the process of HBV infection. The location and replication of HBV in renal tissue make the kidney a potential reservoir for HBV. Previous studies have shown that a high proportion of patients chronically infected with HBV had HBV DNA detected by PCR in their urine. As a result, urine should be considered as a potential route of horizontal and nosocomial transmission of HBV. Our study also suggests that the detection of HBV cccDNA in tissues could serve as a useful tool in the development of anti-HBV drugs, as how to eliminate HBV cccDNA is a key point in the search for such drugs.

Acknowledgments

This study was supported by a grant from the Hunan Ministry of Science and Technology (NO.04SK3044-1), China. We thank the patients and staff of the participating hospitals in Changsha and Yiyang, China for their support. Dr Wen is a recipient of the Ontario Women’s Health Council—Institute of Gender and Health of CIHR Mid Career Award.

Ethical approval: This study was approved by the Research Ethics Board of the Central South University.

Conflict of interest: No conflict of interest to declare.

References