Focal segmental glomerulosclerosis as a complication of hepatitis B virus infection

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Abstract
Human hepatitis B virus (HBV) is well known as a cause of membranous nephropathy (MN). While the association of HBV infection with MN is strong, data regarding its association with other glomerular diseases are conflicting. Here, we report a case of focal segmental glomerulosclerosis (FSGS) with HBV infection. In this case, we have found HBV-DNA in urinary podocytes by real-time PCR methods. After the administration of anti-viral therapy, FSGS improved, paralleling the decreased level of HBV-DNA in podocytes. The refractory FSGS induced by HBV could be effectively treated with appropriate anti-viral agents.

Keywords: anti-viral therapy; focal segmental glomerulosclerosis; human hepatitis B virus

Introduction
A variety of extrahepatic manifestations, one of the commonest being hepatitis B virus (HBV)-associated nephropathy, may appear in persons chronically infected with HBV [1]. HBV is well known as a cause of membranous nephropathy (MN). Several morphological forms of renal disease including MN, membranoproliferative glomerulonephritis (MPGN), IgA nephropathy and rarely focal segmental glomerular sclerosis (FSGS) have been described in association with HBV infection. While the association of HBV infection with MN is strong, data regarding its association with other glomerular diseases are conflicting [1,2].

We report herein a case of FSGS with immunotolerated perinatal HBV infection. It was known that the key event in the pathogenesis of FSGS is podocyte injury [3]. In this case, we have found HBV-DNA in podocytes using real-time PCR methods. After the administration of anti-viral therapy, FSGS improved, paralleling the decreased level of HBV-DNA in podocytes.

Case report
A 33-year-old man was admitted to a hospital because of severe peripheral oedema. Laboratory data showed hypo-
proteinaemia and mild renal failure. Urinary protein was >10 g/day, and a diagnosis of nephrotic syndrome was made. Renal biopsy showed segmental glomerular tuft collapse and overlying visceral epithelial cell hyperplasia and hypertrophy (Figure 1, left). On this basis, a diagnosis of the collapsing variant of FSGS was made. Because hepatitis B surface antigen (HBsAg) and hepatitis B envelope antigen (HBeAg) were detected in the serum, the anti-viral drug lamivudine (25 mg/day) was started before administering prednisolone. Two months later, intravenous methylprednisolone (500 mg × 3 days) was administered. Subsequently, prednisolone at 10 mg/day was introduced. However, proteinuria did not improve. The patient was referred to our hospital 3 months after the onset of disease.

He was normotensive and weighed 72 kg (usual weight was 58 kg) with a height of 160 cm. Investigations showed severe hypoalbuminaemia (serum albumin 1.4 g/dL), renal insufficiency (serum creatinine 2.20 mg/dL) and severe proteinuria (12 g/day). His serum ALT and AST levels were normal. Viral serology was positive for both HBsAg and HBeAg, but negative for HBe antibody (HBeAb). His mother and five of six siblings were positive for HBsAg. These findings indicated immunotolerated perinatal HBV infection. He was negative for human immunodeficiency virus (HIV) and human hepatitis C virus (HCV).

On his admission to our hospital, HBV-DNA analysis showed >10^7.6 copies/mL in the serum. Because lamivudine was not effective against this patient’s HBV, entecavir was considered as an alternative. After administration of entecavir (0.5 mg/day), proteinuria gradually resolved. After 2 months, the patient achieved remission of proteinuria. Further investigations at this time showed serum HBV at 10^7.4 copies/mL, approximately two logs less than the level before entecavir therapy (Table 1). Repeated renal biopsies were taken to confirm the efficacy of entecavir, showing no obvious FSGS lesions in >20 glomeruli (Figure 1, right).

Recent studies have shown that the key event in the pathogenesis of FSGS is podocyte injury [3]. FSGS can occur in patients infected with HIV, when it is known as HIV-associated nephropathy (HIVAN) [4]. Podocyte-restricted expression of HIV gene products is sufficient for the development of HIVAN [5]. It is possible that similar mechanisms of podocyte damage as a result of HBV infection may induce the development of FSGS in HBV-infected individuals. To investigate HBV infection in podocytes, we collected urinary podocytes before and after entecavir therapy. For the isolation of podocytes, we used a monoclonal antibody, which reacted with carbohydrate moiety of extra-domain of podocalyxin (22A4, which was a kind gift from Dr Masanori Hara). Podocyte isolation was performed using the immunobeads method. Subsequently, real-time quantitative PCR analysis was performed [6]. Before the administration of entecavir, the HBV-DNA level in podocytes was 700 copies/μg DNA, decreasing to below the limit of detection on treatment. We also confirmed that HBV-DNA could not be detected in the supernatant nor in sediment of urine, which did not contain podocytes (Table 1).

**Table 1.** The changes of HBV-DNA levels in urine and serum, urinary protein, and numbers of podocytes in urine

<table>
<thead>
<tr>
<th></th>
<th>Pre-entecavir therapy</th>
<th>1 month later</th>
<th>2 months later</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV-DNA in podocytes</td>
<td>700</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>HBV-DNA in serum (copy/μg DNA)</td>
<td>&gt;10^7.6</td>
<td>10^6.9</td>
<td>10^6.4</td>
</tr>
<tr>
<td>Urinary protein (g/24 h)</td>
<td>5.0</td>
<td>4.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Numbers of podocytes in urine (/mL)</td>
<td>3.6</td>
<td>2.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Discussion

FSGS is a pattern of glomerular injury, most commonly associated with idiopathic nephrotic syndrome in adults, and is the most common pattern of glomerular involvement in childhood steroid-resistant nephrotic syndrome.
HIVAN is characterized by collapsing FSGS [4]. Though mechanisms by which HIV-1 genes cause nephropathy are not fully known, transgenic rodent models have shown that podocyte-restricted expression of HIV-1 gene products is sufficient for the development of collapsing FSGS [5]. Several reports indicate a role for parvovirus B19 in collapsing FSGS [7]. Parvovirus B19 DNA was also identified in podocytes of patients with FSGS [7]. Collapsing FSGS is a proliferative disease defined by segmental or global wrinkling of the glomerular basement membranes associated with podocyte proliferation [8]. The pathological similarity of FSGS to HBV infection indicates the same mechanism of HIVAN and parvovirus B19-associated nephropathy.

The diagnosis of HBV-associated glomerulonephritis is established by serologic evidence of HBV antigen or antibodies, by presence of glomerulonephritis on kidney biopsy and by demonstration of one or more HBV-related antigens by immunohistochemistry [1]. There are seven cases reported previously, which showed FSGS complicated by HBV infection [2]. Some of them demonstrated HBV antigen in glomeruli or tubular cells by immunostaining technique [2]. However, there is no report which proved HBV antigens or DNA in podocytes. In our case, we used a quite different technique to prove the HBV-DNA in podocytes. After the entecavir therapy, HBV-DNA level in the extraction of podocytes was decreased below the limit of detection. Proteinuria, renal function and pathological findings dramatically improved, paralleling the decreased level of HBV-DNA. The fact that clinical and pathological findings improved paralleling the decreased level of HBV-DNA in podocytes, suggests that HBV infection of these cells could have been responsible for inducing FSGS in this case. This is the first report documenting HBV infection in podocytes. We conclude that refractory FSGS induced by HBV can be effectively treated with appropriate anti-viral agents.

Conflict of interest statement. None declared.

References

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Atypical presentation of atypical amyloid

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Abstract
Amyloidosis is a group of diseases categorized by precipitation of a group of protein aggregates (amyloid) in tissues, including the kidney, and proteinuria is usually the commonest, though not exclusive, hallmark of clinical presentation. AL and AA are the most commonly recognized forms of amyloidosis involving the kidney, but other forms have been described. We present a case of renal amyloid-