Detection of the hepatitis C virus antigen in kidney tissue from infected patients with various glomerulonephritis

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Abstract

Background. Several studies have postulated a causal link between hepatitis C virus (HCV) infection and renal diseases through the induction of cryoglobulinaemia. However, the detection of viral antigens within kidneys of HCV-infected patients has proved to be difficult. We studied a cohort of Chinese HCV-infected patients with various glomerulonephritis (GN) in an attempt to detect HCV antigens within their kidneys.

Methods. Twenty-one patients with various GN were found to be serum HCV antibody positive (seven serum HCV-RNA positive simultaneously), at the time of renal biopsy, from January 2005 to April 2008 in our hospital. A murine monoclonal antibody against the HCV-NS3 protein was employed to detect the HCV antigen using immunohistochemistry and immunogold labelling. Their clinical and pathological data were collected and further analysed.

Results. The HCV-NS3 antigen was detected in six (6/21, 28.6%) HCV-antibody-positive patients by immunohistochemistry and four out of the six were serum HCV-RNA positive (4/7 in HCV-RNA positive, 57.1%). The HCV antigen mainly displayed a linear or granular deposition along glomerular capillary walls and/or mesangial region. Immunoelectron microscopy showed that the labelling of HCV-NS3 was localized mainly in electronic dense deposits. In the HCV-NS3 detectable patients, three patients were with membranoproliferative glomerulonephritis (MPGN), one with membranous nephropathy, one with IgA nephropathy and one with amyloid nephropathy. The age and urinary protein were significantly greater in HCV-NS3-positive patients than those in HCV-NS3 negative, while serum C3 level was significantly lower in the former group. No significant difference was found in serum ALT, albumin and creatinine level between the two groups.

Conclusion. HCV-NS3 antigens could be detected in kidney tissue of HCV-infected patients with various GN, but mainly in those with MPGN and HCV-RNA positive. HCV itself might be involved directly in the pathogenesis of HCV-associated GN.

Keywords: glomerulonephritis; hepatitis C virus; immunogold labelling; immunohistochemistry; NS3

Introduction

Since its identification in 1989, hepatitis C virus (HCV), a single-stranded RNA virus belonging to the family of Flaviviridae, has become a major public concern because of the high rate of chronic infection and its association with cirrhosis and hepatocellular carcinoma [1]. According to a report of World Healthy Organization, ~170 million people, an estimated 3% of the world population, are infected with HCV [2]. Besides, chronic HCV infection has been related to some extrahepatic disorders, including cryoglobulinaemia, glomerulonephritis (GN), porphyria cutanea tarda, lichen planus, seronegative arthritis, keratoconjunctivitis sicca, Mooren’s corneal ulcer and lymphoproliferative disorders [3]. Johnson et al. [4] first reported eight HCV-infected patients who presented with membranoproliferative glomerulonephritis (MPGN). Thus, a relationship between HCV and GN has been described. Further studies demonstrated that HCV-associated GN was usually in the context of mixed cryoglobulinaemia, especially type II, in which circulating cryoglobulins consist of polyclonal IgG complexed with monoclonal IgM endowed with rheumatoid factor (RF) [5]. However, a direct link between the HCV infection and the nephropathy has yet to be fully defined in view of the difficulty in identifying the HCV antigens within the kidneys [3,6,7]. Therefore, we tried to identify the HCV antigen by immunohistochemistry and immunogold labelling within their kidneys of HCV-infected individuals with various GN, and their clinical and histological associations were further analysed.

Subjects and methods

Patients

Twenty-one HCV-infected patients, with various biopsy-proven GN in Peking University First Hospital, were recruited in the current study.
according to the previously described diagnostic criterion [8]. Nineteen were male and two were female with an average age of 45.6 ± 13.43 (range 20–70) years. All patients were serologically negative for HBV and HIV. None had clinical or biochemical evidence of autoimmune diseases. Upon renal biopsy, all patients had positive serum anti-HCV antibody that was screened by chemiluminescent microparticle immunoassay (CMI) (Ortho-Clinical Diagnostics, Johnson-Johnson Company, Rochester, NY, USA). Although all patients were serum HCV-RNA positive (>1000 copies/mL) in their past history, detected by fluorescence polymerase chain reaction (PCR) (PCR diagnostic, GeneAmp5700, ABI, Foster City, CA, USA), only seven were still positive upon renal biopsy. The clinical and histological data of the 21 patients were collected and further analysed.

**HCV genotyping**

HCV genotyping was performed for the seven HCV-RNA-positive patients. Serum RNA extracting and RT-PCR has been described elsewhere [9]. Amplification of 10 µL cDNA was performed in reaction mixture containing 1 µL 10 × PCR buffer, 2.5 µL dNTPs (Takara, Kyoto, Japan), 0.2 µL Taq DNA polymerase (Takara), 31.8 µL dH2O, 1 µL outer primer (sense 5′-CTG TGA GGA ACT ACT GTT-3′), and 1 µL outer primer (antisense 5′-AAC ACT ACT CGG CTA GCA GTT-3′). This was carried out in a Taqman PCR diagnostic (GeneAmp5700) for 30 cycles using the following program: denaturation for 30 s at 94℃, annealing for 30 s at 55℃ and extension for 2 min at 72℃. A second PCR with nested inner primer (sense 5′-CGGGGAGCTCGCAAGCACCCTAT-3′) and anti-sense 5′-GTCGTGGTACCTCCAGGACC-3′ was performed using 5 µL of the first PCR product. The amplification cycles were identical to the previous one. The second PCR product was then cut with enzymes HaeIII (Promega, Madison, WI, USA). Clones were analysed by electrophoresis using 2% agarose gel (CLP, San Diego, CA, USA).

**Routine renal pathology**

Renal paraffin sections were stained for light microscopy with haematoxylin and eosin, periodic acid-Schiff, Massow trichrome and periodic acid-silver methenamine. IgG, IgA, IgM, C3 and Clq (DAKO A/S, Glostrup, Denmark) were detected with direct immunofluorescence on frozen tissue. The fluorescence intensity was determined using a semi-quantitative scale of 0–4+. Renal ultrastructure was examined using a JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan).

**Detection of HCV-NS3 in renal and hepatic tissue by immunohistochemistry**

Immunohistochemical staining was performed to detect HCV-NS3 on immunohistochemistry Detection of HCV-NS3 in renal and hepatic tissue by using the accessory monoclonal anti-human HCV-NS3 antibody (Millipore, Temecula, CA, USA) as a primary antibody. The sections were deparaffinized in xylene–ethanol at room temperature (RT) and rehydrated in 0.01 mol/L phosphate buffered saline (PBS) immediately before protease treatment. The renal sections were then treated with 0.4% pepsin (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 30 min and hepatic for 20 min. Digestion was terminated by repeated washings in PBS. The sections were immersed into freshly prepared 3% hydrogen peroxide in a methanol solution for 20 min at RT to quench endogenous peroxidase activity. To block non-specific staining, the sections were incubated with 2% bovine serum albumin (BSA) in PBS at 37°C for 30 min. The primary antibody, diluted at 1:200 in PBS, was added on each section directly and incubated overnight at 4°C. The detection system used, PV-9000 (Zhongshan Golden Bridge Biotechnology), was an avidin-free two-step indirect method with anti-mouse IgG conjugated with horseradish peroxidase (HRP) as a secondary antibody. The secondary antibody was incubated for 50 min at 37°C. Then, the sections were washed in drops of 0.05 M Tris–NaCl-buffered saline (pH 7.4) for 1 min. Finally, the sections were counterstained with haematoxylin.

Liver tissue, far apart from tumour, from a 52-year male patient with HCV-associated hepatic carcinoma was used as positive control in which HCV-RNA had been detected previously by in situ hybridization. Renal biopsy specimens from 14 patients (nine males and five females, aged 49.50 ± 9.45 years), with negative serum HCV-RNA and anti-HCV antibody, were used as negative controls, including five patients with idiopathic MPGN, five with membranoproliferative glomerulonephritis (MPGN), two with IgA nephropathy and two with non-IgA mesangial proliferative glomerulonephritis (MsPGN). Renal tissue, obtained from the normal portion of a nephrectomized kidney due to renal carcinoma, was used as normal control.

The adjacent tissue sections were tested by the same procedure as used in negative controls for which the primary antibody was absorbed by preincubation with the recombinant NS3 antigen (Meridian Life Science, Saco, ME, USA) at 37°C for 2 h. In the absorption experiments, the primary antibody was also preincubated with a peptide (sequence: eaikggrhlifchkkkcd, Sangon Biological Engineering Technology, Shanghai, China) that is different from the specific target, but belonging to the NS3 protein. In addition, tissue sections adjacent to the sections with the positive results were stained with mouse monoclonal IgG1 (SouthernBiotech, Birmingham, AL, USA) instead of the mouse monoclonal anti-NS3 (the isotype is IgG1). The immunostaining was also done without the primary antibody.

**Immunoelectron microscopy**

The fixation and preparation of renal specimens for immunoelectron microscopic examination were performed according to previously described procedures [10]. In brief, tissue was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, and then dehydrated and embedded in Epon 812. Ultrathin sections were cut at a thickness of 80 nm and placed on nickel grids. The sections were jet-washed in 0.02 M TBS (pH 8.2) containing 1% bovine serum albumin (w/v) and 0.05% Triton-100 (w/v) and 10% normal goat serum (2% TBSA) for 10 min to exclude non-specific staining, the sections were incubated overnight at 4°C with the same mouse monoclonal anti-human HCV-NS3 (Genetix, MA, dilution 1:150 in 1% TBSA (pH 7.4). After washing in 0.05 M TBS (pH 7.4) and then washing again in 0.02 M TBS (pH 8.2), the sections were incubated in 0.02 M TBS (pH 8.2) containing 1% bovine serum albumin (w/v) and 0.05% Triton-100 (w/v) and 5% normal goat serum (1% TBSA) for 10 min, followed by incubation for 2 h at RT with 10 nm gold-coupled goat anti-mouse IgG (Sigma, St. Louis, MO, USA), diluted 1:40 in 1% TBSA (pH 8.2). The sections were then jet-washed in 0.02 M TBS (pH 8.2) and then in distilled water. After drying, the sections were stained with uranyl acetate. The above liver tissue for immunohistochemistry was used as positive control. Negative controls were performed by replacing the primary antibody with mouse monoclonal IgG1 (SouthernBiotech). The sections were examined at 60 kV using a JEM-1230 electron microscope (JEOL).

**Statistics**

Comparison of quantitative parameters was assessed using the independent samples t-test (for data that were normally distributed) or non-parametric test (for data that were not normally distributed). It was considered a significant difference if the P-value was <0.05. Analysis was performed using the SPSS statistical software package (version 11.0, Chicago, IL, USA).

**Results**

**Routine renal pathology**

The pathological patterns of the 21 patients were as follows: MPGN in six (28.6%) patients, while MN in five (23.8%), focal proliferative IgA nephropathy in four (19.0%), proliferative sclerosing GN or sclerosing GN in three (14.3%), MsPGN in two (9.5%) and amyloid nephrathy in one (4.8%).

**Immunohistochemistry**

Six of the 21 (28.6%) patients were HCV-NS3 antigen positive in their kidney specimens. The HCV-NS3 antigen mainly displayed a linear or granular deposition along glomerular capillary walls and/or mesangial region. In addition, the HCV-NS3 antigen could be detected in a few epithelial cells of the tubules and within some arteriole
HCV antigen in kidney from patients with glomerulonephritis

Fig. 1. The pattern of HCV-NS3 antigen deposition in renal and hepatic specimens from negative control, positive control, disease control and HCV-associated GN. (A) HCV-NS3 positive staining in a section of positive control, mainly be identified in the cytoplasm of hepatocytes. Magnification ×200. Inset ×400. (B) Tissue section adjacent to section shown in (A) as the hepatic negative control, for which anti-NS3 was omitted by preincubation with the recombinant NS3 protein. The positive staining disappeared after preincubation. Magnification ×200. (C) The positive staining could also be observed in the cytoplasm of hepatocytes in the tissue section adjacent to section shown in (A), for which anti-NS3 was preincubated with the peptide which is different from the specific target, but belonging to the NS3 protein. Magnification ×200. Inset ×400. (D) HCV-NS3 negative staining in a section of a MPGN disease control. Magnification ×200. (E) HCV-NS3 deposited mainly in the glomeruli. Magnification ×100. (F) Tissue section adjacent to section shown in (E) as the renal negative control, for which the primary antibody was replaced by mouse monoclonal IgG1. The positive staining disappeared in this section. Magnification ×100. (G−J) A linear or granular deposition along glomerular capillary walls and/or mesangial region from patients of GN. The pathological types of G and J were IgA nephropathy, MPGN, membranous nephropathy and amyloid nephropathy, respectively. Magnification ×200. Inset ×400. (K) HCV-NS3 antigen was detected within some arteriole walls (arrowhead). Magnification ×200. (L) HCV-NS3 antigen was detected in a few epithelial cells of the tubules (arrowhead). Magnification ×200.
walls. Each positive hepatocyte was strongly stained in the cytoplasm. Preincubation with HCV-NS3 recombinant antigens completely abolished the positive signal. Absorption with the peptide that was different from the specific target but belonged to the NS3 protein had no significant effect. No positive reaction was found after immunostaining with mouse monoclonal IgG1 (Figure 1).

In the six patients with positive HCV-NS3 staining, the pathological types were composed of MPGN (3/6, 50.0%), MN (1/6, 16.7%), focal proliferative IgA nephropathy (1/6, 16.7%) and renal amyloidosis (1/6, 16.7%).

**Immunoelectron and electron microscopy**

Immunoelectron microscopy showed that gold particles mainly deposited in cytoplasm of hepatocytes and in immune dense deposits in four of the six HCV-NS3-positive patients, while the gold particles were not observed when the primary antibody was replaced by mouse monoclonal IgG1 (Figure 2). Electron microscopy also revealed microtubular and fibril-like structures (5–35 nm) in two HCV-NS3 detectable MPGN patients that were regarded as cryoglobulins. Amyloid-like fibres (7–10 nm) were identified in one HCV-NS3 detectable patient who presented with renal amyloidosis (Figure 2).

The deposition of the HCV-NS3 antigen, immunoglobulins, complements and electron microscopy profiles of the six patients are presented in Table 1.

**HCV genotyping of patients with positive HCV-RNA**

In the seven patients with positive HCV-RNA, two patients (2/7, 28.6%) were genotype 2a and five patients (5/7, 71.4%) were genotype 1b.

**Clinical manifestations of patients with and without HCV-NS3 staining**

Among the six patients with positive HCV-NS3 staining, four (66.7%) had nephrotic syndrome and three of the four also had microscopic haematuria, while the other two (33.3%) were diagnosed as chronic GN and one with chronic renal failure. Serum cryoglobulin was examined in four patients, one was positive with type II mixed cryoglobulinaemia. HCV-RNA was detected in the cryoglobulin of this patient. Hypocomplementaemia was found in all six patients. RF was examined in four patients; two patients had higher levels of RF. One patient had extra-renal involvement, manifested as palpable purpura and arthralgia.

Among the 15 patients with negative HCV-NS3 staining, proteinuria and microhaematuria were the main manifestations. Eight of the 15 were with nephrotic syndrome, six with chronic nephritis, four with chronic renal insufficiency and one with acute renal injury. A cryoglobulin test was performed in only five patients and two were positive. Hypocomplementaemia was presented in eight (53%) patients. RF was examined in six patients; two patients had higher levels of RF. One patient had extra-renal clinical manifestations, presented as weakness and Raynaud phenomena.

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Fig. 2. (A–C) Electron microscopic examination. (D–H) The localization of the HCV-NS3 antigen in hepatocytes and glomeruli by the immunogold technique. The specimens of A and F were from one patient with MPGN, while C and H were from one patient with amyloid nephropathy, B and G were from patients with MPGN and MN, respectively. The specimen of D was from the liver tissue of a HCV infection patient. (A) The organized deposits under the glomerular endothelium along the glomerular basement membrane with a microtubular appearance. Magnification ×40 000. (B) The fibril-like objects in the matrix of mesangial region (arrowhead). Magnification ×8000. Inset ×60 000. (C) The amyloid-like objects in the mesangial region (arrowhead) with fine filaments appearance. Magnification ×10 000. Inset ×80 000. (D) Immunogold labelling (10 nm) for HCV-NS3 (arrowhead) in the cytoplasmic of hepatocyte. Magnification ×10 000. Inset ×80 000. (E) The labelling for a section from MPGN patients as negative control, for which the primary antibody was replaced by a mouse monoclonal IgG1. Magnification ×60 000. (F) Immunogold labelling (10 nm) for HCV-NS3 (arrowhead) in the mesangial region (arrowhead) with fine filaments appearance. Magnification ×60 000. Inset ×60 000. (G) The amyloid-like objects in the mesangial region (arrowhead) with fine filaments appearance. Magnification ×60 000. Inset ×12 000. (H) Gold particles in the mesangial electron-dense deposits. Magnification ×8000. Inset ×120 000.
Table 1. The pathological features of patients with HCV-NS3 staining

<table>
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<tr>
<td>HCV-NS3 expression</td>
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<tr>
<td>GCW</td>
<td>+</td>
</tr>
<tr>
<td>Ms</td>
<td>+</td>
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<td>IgG</td>
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<td></td>
<td>IgA</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
</tr>
<tr>
<td></td>
<td>C3</td>
</tr>
<tr>
<td>Main location under EM</td>
<td>In GCW and Ms</td>
</tr>
<tr>
<td>Organized deposits</td>
<td>−</td>
</tr>
<tr>
<td>Location</td>
<td>In sub-endo, sub-epi and Ms</td>
</tr>
<tr>
<td>Structure</td>
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<tr>
<td>Immunogold labelling</td>
<td>+</td>
</tr>
<tr>
<td>Main location</td>
<td>In ED</td>
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MPGN, membranoproliferative glomerulonephritis; MN, membranous nephropathy; IgAN, IgA nephropathy; GCW, glomerular capillary walls; Ms, mesangium; sub-endo, sub-endothelium; sub-epi, sub-epithelium; ED, electron-dense deposits.

Table 2. The comparison of clinical characteristics between patients with and without HCV-NS3 staining

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive HCV-NS3 staining</th>
<th>Negative HCV-NS3 staining</th>
<th>P-value</th>
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<tr>
<td>Cases</td>
<td>6</td>
<td>15</td>
<td>0.35</td>
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<tr>
<td>Gender (male/female)</td>
<td>6/0</td>
<td>13/2</td>
<td>0.04</td>
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<tr>
<td>Age</td>
<td>54.83 ± 14.16</td>
<td>41.93 ± 11.62</td>
<td>0.01</td>
</tr>
<tr>
<td>Proteinuria (0–0.15 g/24 h)</td>
<td>7.47 ± 3.76</td>
<td>3.86 ± 2.25</td>
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<td>Serum albumin (35–50 g/L)</td>
<td>25.03 ± 8.46</td>
<td>29.76 ± 9.22</td>
<td>0.95</td>
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<td>ALT (0–40 IU/L)</td>
<td>44.33 ± 11.22</td>
<td>43.73 ± 23.20</td>
<td>0.42</td>
</tr>
<tr>
<td>Serum creatinine (44–133 µmol/L)</td>
<td>144.93 ± 75.36</td>
<td>176.89 ± 167.75</td>
<td>0.01</td>
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<tr>
<td>Serum C3 (0.85–1.13 g/L)</td>
<td>0.60 ± 0.19</td>
<td>0.92 ± 0.24</td>
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ALT, alanine aminotransferase.

Comparison between patients with and without HCV-NS3 staining

The clinical data of the two groups are listed in Table 2. Both were male preponderant. Patients with positive HCV-NS3 staining were much elder (54.83 ± 14.16 versus 41.93 ± 11.62 years, P < 0.05). The level of urinary protein was significantly higher in patients with positive HCV-NS3 staining than those with negative staining (7.47 ± 3.76 versus 3.86 ± 2.21 g/24 h, P < 0.05), while serum C3 level was significantly lower in patients with positive HCV-NS3 staining than those with negative staining (0.60 ± 0.19 versus 0.92 ± 0.24 g/L, P < 0.05). No significant difference was found in serum ALT, albumin and creatinine level between the two groups.

Among the seven patients with positive serum HCV-RNA at the time of renal biopsy, four (57.1%) had positive HCV-NS3 staining in their kidney. The clinical, pathological data of the seven patients are listed in Table 3. The HCV-RNA quantitation was not found to be significantly different between patients with or without HCV-NS3 staining (10^{5.36} ± 1.37 versus 10^{5.23} ± 1.54, P > 0.05).

Discussion

To our knowledge, the diagnostic criterion for defining HCV-associated GN was difficult to establish because of the difficulty in detecting HCV in the renal specimens. It is not clear whether the two diseases develop independently or with a causal link. Therefore, detecting HCV antigens in kidney tissue is crucial to settle this. NS3 is a conservative non-structure component of HCV genome. The molecular weight of the NS3 protein is 52kD. NS3 comprises two domains, one possessing a serine protease activity and the other an RNA helicase activity, both of which are essential for virus replication [11]. In our study, the HCV-NS3 antigen, detected in kidney specimens of six patients with various glomerular disorders, mainly displayed a linear or granular deposition along glomerular capillary walls and/or mesangial region. Immunoelectron microscopy demonstrated that the HCV-NS3 antigen was localized mainly in the electron-dense deposits. Thus, our finding provides convincing evidence for a diagnosis of HCV-associated GN. The success detection and localization of the viral antigen
in the kidney is in agreement with a few previous studies [12,13]. Our current study revealed that the HCV-NS3 antigen was co-deposited with IgG, IgM and C3 in damaged glomeruli; these might support a HCV-associated immune-mediated mechanism within the area of tissue damage.

Although all 21 patients were serum anti-HCV antibody positive, only seven patients were simultaneously serum HCV-RNA positive upon renal biopsy and the HCV-NS3 antigen was detected in four out of seven (57.1%) patients, whereas only 2 out of the 14 (14.3%) HCV-RNA-negative patients were positive for the renal HCV-NS3 antigen. Therefore, serum HCV-RNA positive could increase the likelihood of renal deposition of the HCV antigen, though HCV-RNA level was not associated with HCV antigen deposition in kidney [12]. The two HCV-antigen-detected but serum HCV-RNA-negative patients had >10 years of HCV infection and were serum HCV-RNA positive in the past history. The chronic HCV infection had stimulated production of polyclonal anti-HCV antibodies and monoclonal RF, which combined with the antigens to form immune complex deposits in glomeruli. The HCV antigen would be expected to persist in tissue for some time after clearance of circulating HCV-RNA by interferon-alpha therapy. This could be why we detected the HCV antigen in the two serum HCV-RNA-negative patients.

In comparison with HCV-NS3-negative patients, those with positive staining were much older, which might be associated with the chronic nature of HCV infection. It is suggested that most patients with HCV infection may develop liver cirrhosis, hepatocellular carcinoma and extrahepatic manifestations within 20 years after infection [14]. In addition, patients with positive HCV-NS3 staining had greater proteinuria and lower serum C3 level. These indicated a pronounced renal damage and the subsequent complement activation might play an important role in initiation of HCV-associated GN.

In the current study, the HCV-NS3 antigen could not be detected in kidney specimens of 15/21 patients with HCV infection. However, the absence of detectable NS3 in diseased glomeruli could not completely exclude the diagnosis of HCV-associated GN. Because of the viral genotype and quasi-species diversity of HCV genome [15], the variation of HCV-NS3 might exist in some of these patients. In order to increase the detecting rate, we actually tried three different monoclonal antibodies against HCV, including anti-NS3, anti-core protein and anti-NS5A, to detect HCV antigens. However, the performance of the other two antibodies was unstable, in spite of application of a variety of techniques for antigen retrieval on paraffin-embedded sections. Hence, using combined a panel of stable monoclonal antibodies for detecting HCV antigens requires further investigation.

Renal biopsies of HCV-infected patients displayed a variety of histological patterns. In our study, light microscopy revealed a high incidence of MPGN accounting for 50% of HCV-NS3 positive staining patients. Other forms of glomerular injury that have been previously linked with HCV infection, including MN and IgA nephropathy [16], were also found in our study. The HCV-associated IgA nephropathy was thought to be via immune complex deposits induced by both the HCV antigens and the impaired hepatic clearance of circulating IgA with consequent deposition in the kidney. Furthermore, we found HCV-NS3 positive staining in a patient with renal amyloidosis of AL type, which has not previously been reported in HCV-associated GN. AL amyloidosis is derived from monoclonal immunoglobulin light chain or light chain fragments secreted by a single clone of B cells [17]. HCV has been demonstrated to stimulate monoclonal B cells proliferation through chromosomal rearrangement in HCV-related cryoglobulinaemia [18]. Therefore, it is possible that the proliferation of monoclonal B cell in this case might be related to the result of HCV infection.

HCV have been classified into six main genotypes and a variable number of subtypes within each genotype [19]. The HCV genotype is important as it influences the nature history and antiviral therapy [15]. We found genotypes 2a (2, 28.6%) and 1b (5, 71.4%) in the seven serum HCV-RNA-positive patients, which was consistent with the distribution of HCV genotypes of China, in which the main genotypes are 2a and 1b with 1b preponderant [20]. In the seven patients with positive HCV-RNA, two patients with genotype 2a were HCV-NS3 positive staining, while only two (2/5, 40%) patients with genotype 1b were HCV-NS3 positive staining. In fact, it has been suggested that patients infected with HCV genotype 2 may develop more serious and chronic disease than those infected with genotype 1 [21]. Moreover, patients infected with HCV genotype 2 might have a tendency to develop extrahepatic manifestations including GN than HCV genotype 1 [21].

Hepatitis C is associated most commonly with type II mixed cryoglobulinaemia. Cryoglobulinaemia consists of complexes of RF, IgG, anti-HCV antibodies and HCV. It was reported that testing unselected patients with type II mixed cryoglobulinaemia.
mixed cryoglobulinaemia had shown that up to 90% had anti-HCV antibodies in their serum [22]. In contrast, testing cohorts of patients with hepatitis C infection indicated that cryoglobulins could be detected in up to 50% [23]. The prevalence of cryoglobulinaemia in our series of HCV-infected patients could not be well described because this test was not performed in some patients. However, electron microscopic examination might provide helpful information on the existence of cryoglobulins in kidney sections. In the current study, microtubular and fibril-like structures were found in two HCV-NS3 detectable MPGN patients. Cryoglobulinaemia was confirmed in one patient while the other patient was not screened.

In conclusion, the HCV antigen could be identified in the glomeruli of some HCV-infected patients. HCV itself might be involved directly in the pathogenesis of HCV-associated GN.

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Conflict of interest statement. None declared.

References

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