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Original Article

HBV serum and renal biopsy markers are associated with the clinicopathological characteristics of HBV-associated nephropathy

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Abstract: Background: Accumulated evidence has shown that hepatitis B virus infection is associated with numerous types of nephropathy but it remains to clarify the different role of HBV markers, either in serum or deposit in kidney, in the pathogenesis of HBV-associated nephropathy. In this study, we investigated the relationship between HBV markers and HBV-associated nephropathy by using multi-linear regression in Chinese patients with HBV-associated membranous nephropathy (MN). Methods: A total of 196 cases of HBV-associated MN, which were diagnosed based on renal biopsy, were collected during the period of January 2000 to December 2009 from our hospital. Serum and renal biopsy HBV markers included HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBC. HBV-associated nephropathy was characterized by a panel of clinical manifestations and pathological parameters, which included proteinuria, hematuria, serum creatinine, hypertension, and renal damage in glomeruli, tubules, interstitium, and blood vessels. Multilinear regression was used to analyze the relationship between the HBV markers in serum and renal biopsy and the clinicopathological characteristics of HBV-associated nephropathy. Results: After analysis of the clinical and pathological data in 196 cases of HBV-associated membranous nephropathy, this study revealed that glomerular lesion was marginally associated with serum HBsAg ($P = 0.0528$), Anti-HBs ($P = 0.0978$), but significantly associated with the presence of IgA ($P = 0.0242$), IgG ($P < 0.0001$) and C3 ($P = 0.0064$) in renal biopsy. There was no significant association between glomerular lesion and HBV markers in kidney. The presence of crescent and renal tube impairment was not related to HBV markers. The renal fibrosis was significantly related to gender ($P = 0.023$), age ($P = 0.0211$), HBsAg ($P = 0.0001$) and HBcAg ($P = 0.0083$) and C3 ($P = 0.0299$) in renal biopsy. Notably, the renal blood vessel impairment was significantly related to systolic Blood Pressure (SBP) ($P < 0.0001$), diastolic blood pressure (DBP) ($P = 0.0002$), serum HBsAg ($P = 0.0428$), serum HBeAg ($P = 0.0766$), FRA ($P = 0.0002$), and HBsAg ($P = 0.0241$) and HBcAg ($P = 0.0599$) in renal tissues. Also, the renal interstitial infiltration was related to patient age ($P = 0.015$, SBP ($P < 0.0001$), DBP ($P = 0.0001$), C3 ($P = 0.0028$), FRA ($P = 0.0165$), HBsAg ($P = 0.0016$) and HBcAg ($P = 0.0203$) in kidney biopsy. These results suggest that the major pathological changes in kidneys in HBV patients are related to one or more HBV markers, such as HBsAg, HBeAg, or anti-HBs antibody. Besides, most of the pathological changes in kidneys are related to C3 and FRA in kidney tissues. The clinical markers of nephropathy, such as proteinuria, hematuria and creatine serum levels, were also evaluated for their relationship with HBV markers in serum and kidney tissues. We found proteinuria was marginally related to HBV DNA ($P = 0.0537$), significantly related to IgA (0.0223). Hematouria was significantly related to IgA ($P = 0.0434$), IgG ($P < 0.0001$), and C1q ($P = 0.0282$). The serum creatine level was related to patient gender ($P = 0.0077$), SBP ($P < 0.0001$), DBP (0.0049), IgG (P-0.0006), and C3 ($P = 0.0113$). These clinical manifestations were not related to HBV markers in either serum or kidney. These results indicate that some of clinical manifestations of nephropathy are related to HBV markers, but the relationship is limited.

Keywords: Hepatitis B virus, serum markers, nephropathy

Introduction

Hepatitis B virus (HBV) infection is a worldwide epidemics, and is particularly prevalent in developing countries such as those in Southeast Asia and Africa. In addition to the liver damage, HBV infection causes manifestations in other organs, which is increasingly recognized as a major pathogenesis of HBV-related morbidity and mortality. Amont the extra-hepatic manifestations related to HBV infection, HBV-related nephritis is a major manifestation by

HBV infection [1, 2]. It was reported that HBV-related nephritis has different pathological types, including MPGN, MsPGN, and membranoproliferative glomerulonephritis (MN), etc. MPGN is a chronic progressive glomerulonephritis characterized by subendothelial and mesangial deposition of immune complexes [3], with a high risk of renal failure. MsPGN is manifested with mesangial hypercellularity and/or increase in mesangial matrix [4], with similar clinical manifestation and prognosis to MPGN. MN is a chronic progressive glomerulonephritis that is also characterized by sub-epithelial immune deposits, which causes non-selective proteinuria.

As a common complication of HBV infection, HBV-GN frequently causes renal damage. The serum markers of HBV infection include HBsAg, HBcAg, HBeAg, anti-HBc and anti-HBe antibodies. It was reported that HBsAg and HBcAg were deposited in the glomeruli of nearly half of MPGN and MsPGN patients, and the deposits of HBsAg and HBcAg in glomeruli were correlated with the deposites of IgA, IgG, IgM and C3 in glomeruli.

Zhang et al. [5] proposed that HBV antigen deposit in renal tissue and the glomerular impairment are hallmark of HBV-related nephropathy, and patients with different serum markers of HBV, such as HBsAg-, HBeAg- and anti-HBc-positive patients; HBsAg-, anti-HBe- and anti-HBc-positive patients; HBsAg- and anti-HBc-positive patients; and simple HBsAg-positive patients, may be indirectly associated with the injury and prognosis of HBV-GN.

In this study, we further investigated the relationship between HBV markers and HBV-associated nephropathy, including renal by using multi-linear regression in Chinese population. The present study analysed the changes in HBV markers in serum and in tissues, and as well as the renal functional parameters, clinical manifestations or symptoms and the pathological data in order to define the clinicopathological characteristics of HBV-associated nephropathy.

Methods

Clinical data

A total of 196 patients who were diagnosed as HBV-GN with HBsAg and/or HBcAb deposited in renal biopsy from January 2000 to December 2009, were analysed in this study. There were 144 males and 52 females, with a male-to-female ratio of 2.8:1. The average age of 40.8 ± 14.4 years old, 8.7% patients are less than 20 years of age, 66.3% patients are 21 to 50 years old and 25% patients are more than 51 years of age. The IRB approval was obtained from the ethical committee of China-Japan Friendship Hospital (No: CJFH201212).

Each patient received physical examination and laboratory tests including blood pressure, blood, urine, urine phase contrast microscopy, 24-hour urine protein. The HBV markers (HBsAg, HBcAb, HBeAg, HBsAb, HBeAb) were determined by enzyme-linked immunosorbent assay (Kit provided by the Shanghai Branch of China Industrial Biotechnology Co., Ltd.). To evaluate HBV replication, the HBV-DNA was measured by uantitative PCR, with a sensitivity of < 103 copies/ml, ≥ 105. The Kit was provided by Sun Yat- Da An Gene Co., Ltd.

Kidney histopathology

All patients underwent percutaneous renal biopsy. Each light microscopy specimen was required to include 10 or more glomeruli. HE, PAS, PASM, Masson staining were performed to evaluate the glomeruli, Renal interstitial and the extent of the lesion and the small blood vessels of the kidney. Frozen sections (direct method) were used for renal tissue IgG, IgA, IgM, C3, C4, C1q, HBsAg, HBcAb deposition site and strength. The kit was provided by Beijing Zhongshan Biotechnology Co., Ltd. The immunofluorescence intensity was represented by "+ + +", "+ +", "+", "–", from strong to weak, and negative, respectively.

Statistical analysis

Statistical analysis was performed using SAS Program. Multilinear regression was performed and $P < 0.05$ being considered statistically significant.

Results

General characteristics of patients

A total of 196 cases of HBV-associated MN, which were diagnosed based on renal biopsy, were collected during the period of January 2000 to December 2009 from our hospital.

The incidence ratio of male to female is 2.8:1. The average age of 40.8 ± 14.4 years old, 8.7% patients are less than 20 years of age, 66.3% patients are 21 to 50 years old and 25% patients are more than 51 years of age.

Clinical manifestations and HBV infection

Patients with nephrotic syndrome account for 53.1%. There were 33.2% of patients with urinary protein between 1 g ~ 3 g, 7.1% of patients with urinary protein less than 1 g; 69.4% of patients with hematuria, 3.1% performance gross hematuria. 5.6% of patients with increased serum creatinine levels. Regarding HBV infection and replication, 19.9% of patients were negative of serum hepatitis B antigen and antibody. 30.6% of patients with one or more of serum positive hepatitis B antigen, 45.4% of patients with one or more serum positive hepatitis B antibody; 18.4% of patients with hepatitis B virus DNA replication.

Renal pathological findings

IgG and C3 deposition of glomerular basement membrane was found in 98.5% patients, 37.2% of the patients showed "full house" of immunofluorescence. HBsAg or HBcAg deposition on glomerulus was found in all patients, 79.1% of them were in "double positive" of HBsAg and HBcAg. Light microscopy showed 23.9% of patients were membranous nephropathy (MN) and 76.1% were atypical membranous nephropathy (AMN). In the group of AMN patients, the incidence rate of hematuria (73.8% Vs 55.3%, $P < 0.05$), replication rate of HBV-DNA (20.8% Vs 10.6%, $P < 0.05$) and "full house" of immunofluorescence (40.3% Vs 27.7%, $P < 0.05$) were significantly higher than that in patients with MN, There were no significant differences in gender, age at onset, incidence of nephrotic syndrome and renal HBV antigen staining between MN and AMN group. In 11 patients with renal dysfunction, their renal pathological type was AMN, they all had renal interstitial fibrosis and two of them were found to have crescent formation.

Relationship between HBV markers in serum and kidney tissues and the pathological changes in HBV nephropathy

Among the 196 cases of HBV nephropathy, Glomerular lesion was marginally associated with serum HBsAg ($P = 0.0528$), Anti-HBs ($P = 0.0978$), but significantly associated with the presence of IgA ($P = 0.0242$), IgG ($P < 0.0001$) and C3 ($P = 0.0064$) in renal biopsy. There was no significant association between glomerular lesion and HBV markers in kidney. The tubular injury was related to C3 ($P = 0.0307$) but not found to be associated with serum and renal HBV markers. The renal interstitial infiltration was related to patient age ($P = 0.015$, SBP ($P < 0.0001$), DBP ($P = 0.0001$), C3 ($P = 0.0028$), FRA ($P = 0.0165$), HBsAg ($P = 0.0016$) and HBcAg ($P = 0.0203$) in kidney biopsy. The renal fibrosis was associated with patient gender ($P = 0.023$), age ($P = 0.0211$), SBP ($P < 0.0001$), DBP ($P = 0.0002$), IgG ($P = 0.0111$), C3 ($P = 0.0299$), and FRA ($P = 0.0294$). HBsAg ($P = 0.0001$) and HBcAg ($P = 0.0083$) in kidney biopsy. The renal vascular lesion was associated with patient age ($P = 0.0022$), SBP ($P < 0.0001$), DBP ($P = 0.0002$), serum HBsAg ($P = 0.0428$), serum HbeAg ($P = 0.0766$), RFA ($P = 0.0002$), and HBsAg ($P = 0.0241$) and HBcAg ($P = 0.0599$) in renal tissues. The presence of crescent and renal tube impairment were also not related to HBV markers. These results suggest that the major pathological changes in kidneys in HBV patients are related to one or more HBV markers, such as HBsAg, HBeAg, or anti-HBs antibody. Besides, most of the pathological changes in kidneys are related to C3 and FRA in kidney tissues.

Relationship between HBV markers in serum and kidney tissues and the clinical manifestations of HBV nephropathy

The clinical markers of nephropathy, such as proteinuria, hematuria and creatine serum levels, were also evaluated for their relationship with HBV markers in serum and kidney tissues. We found proteinuria was marginally related to HBV DNA ($P = 0.0537$), significantly related to IgA (0.0223). Hematouria was significantly related to IgA ($P = 0.0434$), IgG ($P < 0.0001$), and C1q ($P = 0.0282$). The serum creatine level was related to patient gender ($P = 0.0077$), SBP ($P < 0.0001$), DBP (0.0049), IgG (P-0.0006), and C3 ($P = 0.0113$). These clinical manifestations were not found to be related to HBV markers in either serum or kidney. These results indicate that some of clinical manifestations of nephropathy are related to HBV markers, but the relationship is limited.

Discussion

In this study, we demonstrated that in HBV-associated nephropathy, the pathological changes in kidney were associated with HBV markers. Specifically, glomerular lesion was marginally associated with serum HBsAg and Anti-HBs, but significantly associated with the presence of IgA ($P = 0.0242$), IgG ($P < 0.0001$) and C3 ($P = 0.0064$) in renal biopsy; the tubular injury was related to C3 but not with serum and renal HBV markers. The renal interstitial infiltration was related to C3, FRA, HBsAg and HBcAg in renal biopsy. The renal fibrosis was associated with IgG, C3, and FRA, and HBsAg and HBcAg in kidney biopsy but not those markers in serum. The renal vascular lesion was associated with HBsAg HbeAg in serum and HBsAg and HBcAg in renal biopsy. It was also associated with RFA in renal biopsy. Based on these results, it appeared that the severity of nephropathy was more associated with HBV markers in renal biopsy rather than in serum. Besides, compared to renal pathological changes, the clinical manifestations of HBV-associated nephropathy were less associated with HBV markers. Our results showed that the clinical manifestations, such as proteinuria and hematouria, were not associated with HBV markers, either in serum or in renal biopsy, but with IgA, IgG, or C1q deposits in renal tissues. This is consistent with previous reports that in patients with chronic hepatitis B and asymptomatic carriage of HBV, the clinical manifestations during the initial several years are usually not specific with relatively normal hepatic and renal functions, however, the renal damage is progressive and irreversible, and associated with high morbidity rate [6].

The relationship between HBV infection and IgA nephropathy has been intensively investigated. It was reported by Lai et al that the morbidity due to IgA nephropathy was positively correlated with HBV prevalence, and HBsAg, HBcAg and the corresponding immune complexes were deposited in the glomeruli of patients with IgA nephropathy. Notably, the deposit site was the same as that of IgA, supporting the role of HBV and its immune complexes in the pathogenesis of IgA nephropathy [7-11]. It has been clear that antigen-antibody immune complexes against HBs, HBc, or HBe together with complement components are able to induce renal damage in chronic HBV infection.

As the most important determinant of viral pathogenesis of HBV, the hepatitis B virus X protein (HBx) was reported to be involved in the damage of renal tubular epithelial cells in patients with HBV-GN [12, 13]. It was reported that HBx can modulate the functions of AP-1 and NF-κB [14], and activate the Ras/Raf/ERKs-, PI3K-Akt- and JAK/STAT-signalling pathways [15, 16], suggesting that HBx can activate immune cells and inflammatory mediators [17], which may play an important role in the progress of HBV-GN.

Although it has been well established that HBV infection is associated with the development of glomerulonephritis, the pathogenic mechanism remains to be fully elucidated, especially how HBx contributes to HBV-GN progression. It has been reported that Notch1 is aberrantly expressed in the renal tissue of patients with HBV-GN, and HBx can upregulate the level of Notch1 mRNA and Notch1 protein production in cultured renal cells. Therefore, Notch1 may play a role in HBV-associated nephropathy [18].

However, the mechanism by which HBV induces HBV-associated nephropathy remains far from being completely understood. Further molecular and clinical case studies are needed to elucidate the pathogenesis.

Disclosure of conflict of interest

None.

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