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Original Article

Correlation between serum hepatitis B virus DNA replication level and clinicopathology in 235 patients with hepatitis B associated glomerulonephritis

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Abstract: Objective: To explore the correlation between serum hepatitis B virus (HBV-DNA) replication levels and clinicopathology in patients with HBV-associated glomerulonephritis (HBV-GN). Methods: A total of 235 patients with HBV-GN were divided into 4 groups, according to the serum titer of HBV-DNA: the viral replication negative group (n = 85), the low-level replication group (n = 35), the moderate-level replication group (n = 38), and the high-level replication group (n = 77). The results of routine blood examination, blood biochemistry, immune globulin, serum complement components C3 and C4, routine urine examination, 24-h urine protein, and other measurements were obtained. The differences between the clinical and pathological manifestations of the different viral replication groups were analyzed by semi-quantitative integration. Results: With increasing serum HBV-DNA level, urinary protein excretion was increased, while albumin in plasma and C3 and C4 in serum were decreased. The differences were statistically significant ($P<0.05$). In addition, with increasing serum HBV-DNA levels, glomerular injury was aggravated in patients with a pathological type of membranous nephropathy, and tubulointerstitial damage became worse in patients with pathological types of IgA nephropathy. There was no significant difference in the degree of pathological HBV markers deposited in renal tissues among the groups with different levels of HBV-DNA replication. Additionally, the serum HBV-DNA level was positively correlated with C4 deposition in renal tissues. Conclusion: With increasing serum HBV-DNA replication levels, clinical and renal pathological manifestations were aggravated in HBV-GN patients.

Keywords: Hepatitis virus, hepatitis B, nephritis, DNA virus, clinical manifestations, pathology

Introduction

Hepatitis B virus (HBV) infection occurs worldwide, especially in southeastern Asia and Africa. It can cause chronic hepatitis, cirrhosis and even liver cancer. In addition, the hepatitis B virus can cause many types of extra-hepatic damage, which have an expanding prevalence [1]. Hepatitis B virus-associated glomerulonephritis (HBV-GN), an immune mediated glomerulonephritis, is a common manifestation of extra-hepatic organ damage [2] caused by hepatitis B virus infection [3, 4]. Studies have shown that clinical and pathological manifestations became worse in HBV-GN patients with a high HBV-DNA load, but most have been small-sample studies [5]. Moreover, previous studies observed that HBV-GN is a common secondary renal disease, that is detected in 5.6% of infected patients' accepted renal biopsies [7]. Based on a clinical retrospective study of 235 cases of HBV-GN, the relationship between the serum HBV-DNA load and the clinical manifestations and pathological changes in HBV-GN was investigated.

Materials and methods

General information: patient criteria

The information on 317 cases of HBV-GN patients who were hospitalized and diagnosed in the Department of Nephrology, PLA General Hospital was collected. The inclusion criteria of the patients were as follows: (1) age from 9 to 70 years; (2) kidney biopsy examination confirmed as carrying hepatitis virus related anti-

gens (including HBsAg, HBcAg, and HBeAg) and a diagnosis of HBV-GN according to pathology; and (3) glomerular filtration rate (eGFR) great than 30 ml/(min.1.73 m^2) according to the Modification of Diet in Renal Disease formula (CKD-MDRD). The exclusion criteria of the patients were as follows: (1) presence of other primary or secondary diseases; and (2) previous treatment with antivirals or immunosuppressants [8]. Among these cases, 235 patients (182 males and 53 females, male-to-female ratio of 3.4:1) with HBV-DNA levels tested were selected, and the average age was 39.4 ± 14.1 (range, 9~65) years old.

Standard for pathological diagnosis

Renal biopsy was performed on all subjects. The biopsy specimens were subjected to hematoxylin and eosin (HE), periodic acid-Schiff (PAS), periodic acid-silver methenamine (PASM), and Masson staining. The frozen tissues were tested for immunoglobulin (IgA, IgG, and IgM), C3, C4, C1q, Fib and hepatitis B surface antigen and core antigen by immunofluorescence. Patients satisfying the following three requirements were diagnosed as having HBV-GN: (1) positive results for hepatitis B virus markers in serum; (2) glomerular nephritis but no secondary glomerular disease such as lupus glomerulonephritis; and (3) hepatitis B virus (HBV) antigen found in the renal tissues.

Experimental groups

Based on the degree of HBV-DNA replication in serum, which was determined by fluorescence quantitative polymerase chain reaction, the 235 HBV-GN patients were divided into four groups, with 85 cases in the negative group (DNA<10^3 copies/ml), 35 cases in the low-level replication group (10^3≤DNA<10^5 copies/ml), 38 cases in the moderate-level replication group (10^5≤DNA<10^7 copies/ml), and 77 cases in the high-level replication group (DNA≥10^7 copies/ml) [6]. There were no significant differences (P>0.05) in age or gender among the four groups of patients. The eGFR for all patients was estimated using MDRD equation. Renal pathology was investigated based on the Katafuchi [6] semi-quantitative integration method. For the patients with membranous nephropathy, another evaluation was added to assess the formation of double contours and spikes. No formation of a double contour or spikes was graded as 0, and the formation of either a double contour or spikes was graded as 1.

Observation index: A blood examination was performed, including alanine aminotransferase, aspartate aminotransferase, total protein and albumin serum creatinine, urea nitrogen and serum uric acid, serum immunoglobulin and complement components C3 and C4, 5 secondary liver indexes, and serum HBV-DNA load. A routine urine examination was also performed, including 24-h urine protein, eGFR, urine NAG enzyme and kidney pathology results.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 software. All measured data are presented as the mean ± standard deviation. Comparisons among groups were performed using a one-way analysis of variance (ANOVA), and comparisons between pairs of groups were examined using the LNK test. Semi-quantitative scoring of renal pathology did not indicate a normal distribution, and the rank-sum test was conducted. Enumerated data were analyzed using the x^2 test. The correlation was analyzed using the Spearman rank correlation test. P<0.05 was considered significantly different.

Results

Comparing clinical manifestations and pathologic types

Nephrotic syndrome was the most common clinical manifestation. With increasing viral DNA load, the proportion of patients with syndrome showed a rising trend, while the proportion with nephritis syndrome showed a decreasing trend (P<0.01), and nephrotic syndrome in the high-level replication group was significantly increased. Almost all patients exhibited proteinuria. There were no significant differences (P>0.05) in the proportion of patients with renal insufficiency (eGFR<60 ml/[min.1.73 m^2]) or hypertension exhibited no significant difference (P>0.05) among the groups. Among the various pathological types, the proportion of membranous nephropathy was the highest, as the same as results before [7-9]. No significant differences in the pathological types of viral replication were identified among the different groups (P>0.05, **Table 1**).

Table 1. The comparison of clinical manifestation and pathology in different groups (n, (%))

Items	Negative group (n=85)	Low group (n=35)	Moderate group (n=38)	High group (n=77)	Total
Gender					
Male	64	27	27	59	182
Female	21	8	11	18	53
Clinical manifestation					
Simple hematuria	1 (1.2)	2 (5.7)	2 (5.3)	1 (1.3)	6 (2.5)
Chronic nephritic syndrome	42 (49.4)	12 (34.3)**	12 (31.6)**	6 (7.8)**	72 (30.6)
Nephrotic syndrome	27 (31.7)	18 (51.4)	19 (50.0)	60 (78.0)	124 (52.8)
Renal insufficiency	15 (17.6)	3 (8.6)*	5 (13.2)*	10 (13.0)*	33 (14.0)
Hypertension	28 (32.9)	12 (34.3)	15 (39.5)	31 (40.2)	86 (36.6)
Pathological types*					
Membranous nephropathy	48 (56.4)	19 (54.3)	25 (65.8)	52 (67.5)	144 (61.3)
IgA nephropathy	24 (28.2)	8 (22.8)	9 (23.7)	6 (7.8)	47 (20.0)
Membranoproliferative glomerulonephritis	10 (11.7)	7 (20.0)	3 (7.9)	19 (24.7)	39 (16.6)

Note: *P>0.05, **P<0.01, compared with the viral replication negative group.

Table 2. The comperatiton of laboratory examination and immunologic test in different groups (x±s)

	Viral replication negative group (n = 85)	Low-level replication group (n = 35)	Moderate-level replication group (n = 38)	High-level replication group (n = 77)
Alanine transaminase (U/L)	23.4±26.8	31.5±19.2**	50.3±47.3**,##	41.7±32.1**,##,¶
Aspartate Transaminase (U/L)	23.3±24.6	28.3±18.7**	55.9±43.2**,##	47.3±44.2**,¶
Total bilirubin (umol/L)	12.0±5.4	11.5±5.4	10.3±6.9	11.0±4.9
Direct bilirubin (umol/L)	3.2±1.3	3.7±3.4	3.6±1.8	3.4±2.0
Gamma-GT (U/L)	47.2±27.9	67.9±51.4	53.6±55.7	76.0±73.7
Total protein (g/L)	53.7±10.4	53.1±11.0	56.5±10.0	58.2±10.1
eGFR (ml/min)	94.7±27.4	97.6±43.4	94.6±38.5	87.1±30.8
Serum creatinine (umol/L)	76.7±30.8	74.9±41.6	84.5±33.8	94.3±44.6
Urea nitrogen (umol/L)	6.5±3.3	7.2±4.3	6.6±4.4	7.5±4.4
Serum albumin (g/L)	32.5±8.4	31.1±6.8	29.4±5.3	29.2±6.9*
Serum uric acid (umol/L)	380.3±101.4	398.4±99.0	362.5±91.5	387.8±103.6
Urine protein (g/L)	1.2±0.9	1.5±0.6**	1.8±0.9**,##	2.7±2.2**,##,¶
Urine NAG enzyme (U/L)	46.5±42.5	42.6±35.7	39.4±22.1	50.787±47.904
IgG (mg/dl)	842.5±336.1	866.4±360.2	823.4±303.5	808.5±375.2
IgA (mg/dl)	261.0±108.7	240.6±114.5	231.4±132.5	224.5±100.7
IgM (mg/dl)	117.0±54.8	122.6±47.8	140.1±56.1	131.0±63.3
C3 (mg/dl)	106.8±34.4	95.0±25.6	93.4±23.1	81.7±26.0**,##
C4 (mg/dl)	24.5±7.8	24.9±8.8	20.7±5.6**,#	17.3±6.2**,##

Note: *P<0.05, **P<0.01, compared with the viral replication negative group; #P<0.05, ##P<0.01, compared with the low-level replication group; ¶P<0.01, compared with the low-level replication group.

Comparing liver and kidney function and immunological parameters

Differences in alanine aminotransferase and aspartate aminotransferase were identified among the groups (P<0.01), and the values were highest in the moderate-level replication group. There were no statistically significant differences in total bilirubin, direct bilirubin, albumin and gamma-GT among the groups (P>0.05). The eGFR, serum creatinine, blood urea nitrogen, serum uric acid and urine NAG enzyme parameters did not differ significantly among the groups (P>0.05). The serum albumin in the high-level replication group was significantly increased (P<0.05) compared with the negative group. With increasing viral load, the amount of urinary protein excretion gradually

Table 3. The comparison of renal pathological score in different groups (median min~max)

	Viral replication negative group (n=85)	Low-level replication group (n=35)	Moderate-level replication group (n=38)	High-level replication group (n=77)
Membranous nephropathy				
Glomerular scores	5.0 (2~8)	5.0 (1~14)	6.0 (2~14)	6.0 (2~14)*,##
Tubular-interstitial scores	2.0 (0~5)	1.0 (0~7)	3.0 (0~7)	3.0 (0~9)
Vascular scores	0 (0~3)	0 (0~1)	0 (0~1)	0 (0~3)
Total scores	7 (1~15)	8.0 (2~13)	8.0 (2~24)	13.0 (1~26)#
IgA nephropathy				
Glomerular scores	4.0 (3~8)	5.0 (4~7)	6.0 (3~12)	7.0 (2~10)
Tubular-interstitial scores	3.0 (0~8)	3.0 (2~5)	5.0 (3~9)**,#	8.0 (4~9)**,#
Vascular scores	0 (0~2)	0 (0~0)	1.0 (0~2)	1 (0~1)
Total scores	10.0 (4~17)	7.0 (6~8)	14.0 (6~20)#	14.0 (6~19)

Note: *P<0.05, **P<0.01, compared with the viral replication negative group; #P<0.05, ##P<0.01, compared with the low-level replication group.

Table 4. The comparison of renal immunofluorescence in different groups (n, (%))

	Viral replication negative group (n = 85)	Low-level replication group (n = 35)	Moderate-level replication group (n = 38)	High-level replication group (n = 77)	Total
C3	66 (77.6)	24 (68.6)	30 (78.9)	58 (75.3)	178 (75.7)
C4	29 (34.1)	18 (51.4)	27 (71.1)*,#	55 (71.4)*,#	129 (54.9)
HBsAg	58 (68.2)	6 (17.1)	13 (34.2)*	31 (40.3)	108 (46.0)
HBcAg	11 (12.9)	11 (31.4)	2 (5.3)	12 (15.6)	37 (15.7)
HBsAg and HBcAg	16 (18.8)	20 (57.1)*	24 (63.2)*	31 (40.3)	90 (38.3)

Note: *P<0.05, compared with the viral replication negative group; #P<0.01.

increased, and the difference among groups was significant (P<0.01). There was no significant difference in IgG, IgA and IgM (P>0.05) among the groups. However, significant differences in serum C3 and C4 were identified among the groups. With increasing HBV-DNA load, serum C3 and C4 were decreased (P<0.01, **Table 2**).

Comparing scores for pathologic membranous nephropathy and IgA nephropathy

With increasing viral replication, pathologic manifestations in patients with membranous nephropathy exhibited a trend toward aggravation. The total score in the high-level replication group was significantly higher than that in the negative and low-level replication groups (P<0.05). The glomerular lesions were aggravated with increasing HBV-DNA load, and there were significant differences in scoring between the high-level replication and negative groups (P<0.05). There were no statistically significant difference in semi-quantitative scoring for tubulointerstitial renal lesions or renal vascular lesions among the groups (P>0.05). With increasing viral replication, semi-quantitative scoring for the renal interstitium and renal tubule exhibited a rising trend in patients with IgA nephropathy. Compared with the negative group, the scores in the moderate-level and high-level replication groups were significantly increased (P<0.05 and P<0.01, respectively). There were no statistically significant differences in semi-quantitative scoring for glomerular disease or renal vascular disease (P>0.05, **Table 3**).

Comparing immunofluorescence in renal tissues

The immunofluorescence of the HBV viral replication marker in the viral replication negative group was mainly due to pure HBsAg deposition, and there were significant differences compared with the moderate-level replication group (P<0.05). There was no significant difference in the positive rate of HBcAg deposition among the groups. The deposition in the low-level and moderate-level replication groups was mainly HBsAg and HbcAg co-deposition, and there were significant differences com-

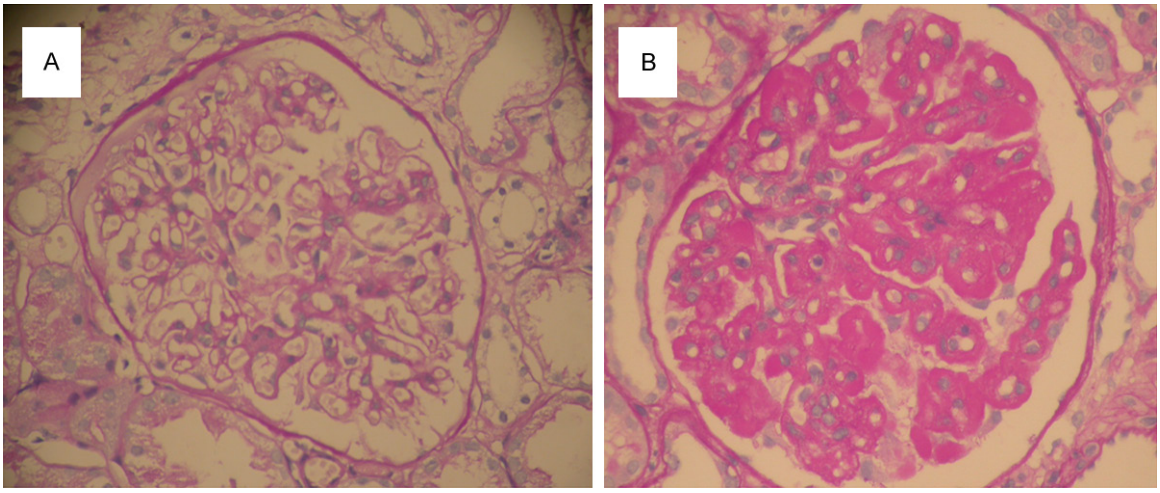

Figure 1. With increasing HBV-DNA replication, the main damage for membranous nephropathy was aggravating glomerular injury (A, B).

pared with the negative group (P<0.05). No significant difference in C3 deposition was identified among the groups. With increasing serum viral replication, there was a significant difference in C4 deposition in renal tissues among the groups (P<0.05, **Table 4**). The higher the serum HBV-DNA, the higher the C4 deposition rate in renal tissues. There was a positive correlation between these two factors (r=0.354, P<0.01). The immunofluorescence renal pathology examination showed that 80.9% (190/235) of HBV-GN patients exhibited HBsAg or HbcAg granular deposition along the capillary loop, and a minority of patients exhibited granular deposition in the mesangial area. The deposition distribution was substantially the same as the distribution of the immunoglobulins and complements. The patients in the viral replication negative and positive groups exhibited 3.53% (3/85) and 14.67% (22/150) altered immune protein deposition ("Full bright"), respectively, such as IgG, C3, C4, C1q, and Fib, for immunofluorescence in renal tissue.

Discussion

The pathogenesis of HBV-GN remains unclear. The possible factors include antigen-antibody complex deposition, direct infection of renal cells, immune system dysfunction and genetic and social factors. Most reports have suggested that the incidence of HBV-GN is mainly due to the antigen-antibody complex deposition in the glomerular capillary loops, resulting in activation of the complements and causing immune injury [3, 4]. Serum HBV-DNA load testing is a key indicator for the clinical observation of viral replication and infection as well as the guidance of clinical treatment [10]. The higher the viral load, the more active the viral replication *in vivo*, which causes more hepatitis B antigen to be released into the blood, resulting in increased formation of the antigen-antibody complex. In patients with non-immune tolerance in terms of the HBV-DNA load, the liver damage is more severe. Accordingly, this paper also showed statistically significant results regarding the impact of the levels of HBV-DNA load on the degree of clinical and pathological manifestations of hepatitis B virus-related glomerulonephritis (HBV-GN).

The results of this study showed that with increasing HBV-DNA load, the clinical manifestations and biochemical indicators changed in patients with HBV-GN, which were detected as increased 24-h urine protein, decreased serum albumin and decreased complements C3 and C4. Combined with analysis of the pathological data, the data indicates that the increased urine protein may be caused by aggravated glomerular injury. Serum albumin may be reduced by the increase in urine protein or by the liver damage caused by increased HBV-DNA, resulting in reduced albumin synthesis. A study by Ren *et al.* [11] showed 520 upregulated genes in HBV-GN mouse kidneys. These genes are primarily related to complement activation, coagulation and acute phase responses. The study also found that mouse serum C3 levels dec-

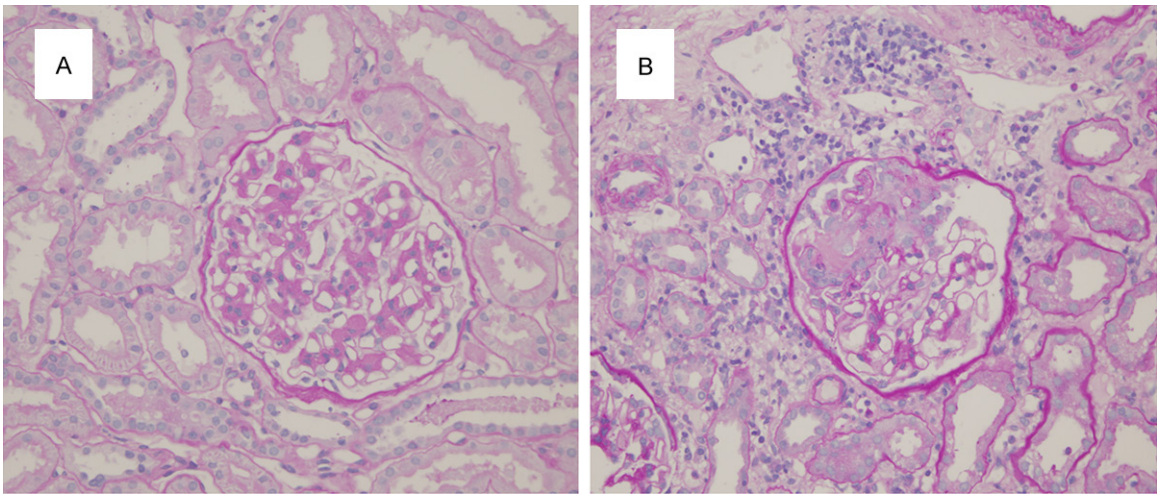

Figure 2. With increasing HBV-DNA replication, the main damage for IgA nephropathy was aggravation of the tubulointerstitial damage (A, B).

reased but kidney C3 secretion increased, suggesting that HBV infection leads to an upregulated complement-mediated inflammatory response pathway in the kidney, which is consistent with this study's findings that with increasing HBV-DNA load, the complement C3 in the blood was decreased.

The results of this study also showed that with increasing HBV-DNA replication, there is an increasing trend of renal pathological damage, but the main damage for membranous nephropathy was aggravating glomerular injury, while the main damage for IgA nephropathy was aggravation of the tubulointerstitial damage (**Figures 1**, **2**). By pathological analysis for patients with HBV-related membranous nephropathy (HBV-MN), Bhimma *et al.* [12] found that most patients exhibited atypical membranous nephropathy, showing that with increasing HBV-DNA load, the basement membrane thickened and the double contour and spikes were aggravated in patients with HBV-GN. These changes may have been caused by increased kidney antigen-antibody immune complex deposition and the activation of many inflammatory cells and inflammatory mediators, which is caused by increasing HBV-DNA and leads to and increased glomerular damage. The literature indicates that the reason elevated HBV-DNA leads to renal tubular interstitial damage in patients with IgA nephropathy may be through hepatitis B antigen expression in the renal tissue of patients with IgA nephropathy infected with HBV, which induces $CD4^+$ and $CD8^+$ cell infiltration and thereby increases the renal tubule interstitial damage. The study found that not all patients in the HBV-DNA negative group had mild pathological damage, suggesting that the complex pathogenesis of HBV may lead to immune system dysfunction, and HBV infection can cause the activation of a variety of autoantibodies [13]. In addition to damage from immune complex deposition, abnormal immune function in patients with HBV-GN can lead to kidney damage [14].

In summary, HBV-DNA replication levels may affect the extent of clinical and renal pathologic damage in patients with HBV-GN and can thus provide a guide for HBV-GN kidney biopsy and clinical antiviral therapy. However, due to the complexity of the pathogenesis and progression of HBV-GN, basic medical research on this topic should be extended. The results of this study need to be confirmed by large-scale multi-center epidemiological studies.

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Disclosure of conflict of interest

None.

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