

# Expression and Significance of Hepatitis B Virus X Protein and Hepatitis B Virus DNA Levels in Renal Tissue of Patients with Glomerulonephritis and Hepatitis B Virus Infection

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## Ma *et al.*: Significance of HBX Protein and HBV-DNA Levels in Patients with Glomerulonephritis and HBV Infection

To investigate the expression and clinical significance of hepatitis B virus X protein and hepatitis B virus deoxyribonucleic acid in hepatitis B virus associated nephritis. 78 patients with hepatitis B virus associated nephritis treated in our hospital from January 2018 to January 2020 were selected as observation group. At the same time, 70 patients with nephritis with positive hepatitis B markers but without hepatitis B surface antigen and/or hepatitis B core antigen deposition in renal tissue were selected as the control group. The clinical data, hepatitis B virus X protein and hepatitis B virus deoxyribonucleic acid were compared between the two groups. There was no significant difference in white blood cells, red blood cells, platelet count test, activated partial thromboplastin time, prothrombin time between the observation group and the control group ( $p > 0.05$ ). The positive rate of hepatitis B virus X protein and the proportion of hepatitis B virus deoxyribonucleic acid  $> 105$  IU/ml in the observation group were 61.54 %, 89.74 % and 52.56 % respectively, which were significantly higher than those in the control group ( $p < 0.05$ ). The proportion of 'Xiaosanyang' and hepatitis B virus deoxyribonucleic acid  $< 103$  IU/ml were 32.05 % and 11.54 % respectively, which were significantly lower than those in the control group ( $p < 0.05$ ). The proportion of hepatitis B virus deoxyribonucleic acid quantification  $> 105$  IU/ml in the observation group was 62.50 %, which was significantly higher than that in the non-three-positive patients ( $p < 0.05$ ). The proportion of patients with hepatitis B virus deoxyribonucleic acid quantification  $> 105$  IU/ml in the observation group was 76.00 %, which was significantly higher than that in patients with effective treatment ( $p < 0.05$ ). The levels of hepatitis B virus X protein and hepatitis B virus deoxyribonucleic acid in patients with hepatitis B virus-associated nephritis were higher and the level of hepatitis B virus deoxyribonucleic acid was related to Sanyang and therapeutic effect, but hepatitis B virus X protein was not significantly correlated.

**Key words:** Hepatitis B virus X protein, hepatitis B virus DNA, hepatitis B virus associated glomerulonephritis, clinical significance

High incidence of hepatitis B virus infection in Chinese people, can cause hepatitis B virus associated nephritis, the specific pathogenesis is not yet fully clear. It has been reported that hepatitis B virus intact virus particles exist in glomeruli and hepatitis B virus DNA (HBV-DNA) can also be detected in glomeruli and renal cortex, suggesting that hepatitis B virus replication has a direct effect on renal tissue<sup>[1]</sup>. There are different clinical reports on evaluation indexes of hepatitis B virus associated nephritis. Hepatitis B virus X (HBX)

protein is encoded by X protein gene and has transactivation effect, which can activate transcription factors to cause nuclear translocation and promote the occurrence and development of inflammation. HBV-DNA is a cyclic DNA molecule with incomplete double strands and its sequence is highly conserved. It is a basic viral protein in the viral infection cycle of the body, mainly located in the host cytoplasm. However, the clinical reports on the changes of two proteins in patients with hepatitis B virus-associated nephritis and

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the evaluation of therapeutic effect are relatively rare<sup>[2]</sup>. In this study, the expression of HBX protein and HBV-DNA in hepatitis B virus-associated glomerulonephritis and the evaluation of therapeutic effect were analyzed. The results are reported as follows. 78 patients with hepatitis B virus associated nephritis treated in our hospital from January 2018 to January 2020 were selected as observation group. At the same time, 70 patients with nephritis with positive hepatitis B markers but without Hepatitis B surface antigen (HBsAg) and/or Hepatitis B core antigen (HBcAg) deposition in renal tissue were selected as the control group. There was no significant difference in gender and age between the observation group and the control group ( $p>0.05$ ). Inclusion criteria-Renal biopsy was performed in our hospital; Age  $>18$  y; Patients and their families informed consent. Exclusion criteria-Combined with hepatitis C virus and other hepatitis virus infection; There are lupus nephritis, purpura nephritis, diabetic nephropathy, tumor associated nephritis, hypertensive nephropathy, etc. HBV-DNA level determination-Take 0.5 mL centrifuge tube to mark and add 100  $\mu$ L cracking liquid that has been fully shaken to each centrifuge tube, then add 50  $\mu$ L specimens or standards to each centrifuge tube, after vortex mixing, placed at room temperature for 10 min; Centrifuge 10 000 rpm for 25 s, pour the supernatant, adding detergent 150  $\mu$ L, fully vortex mixing, again centrifuge 1000 rpm for 25 s, pour the supernatant, again adding repeated detergent 150  $\mu$ L, fully vortex mixing, centrifuge 10 000 rpm for 25 s, discard the supernatant to be measured. Add 30  $\mu$ L reaction buffer to the prepared precipitate and suspension evenly, take 25  $\mu$ L to each reaction tube, suspended evenly and centrifuged slightly, then placed in the fluorescence PCR amplification instrument, cycle conditions: 38° 5min, 95° 10min. Enter the following cycle: 95° 15 s, 58° 50 s (read fluorescence after 30 s), 40 cycles. The automatic medical polymerase chain reaction (PCR) analysis system was used for analysis. The fluorescence signals of 1-10 cycles were selected as the baseline. The setting principle of the threshold was that the threshold line was just slightly higher than the highest point of the amplification curve of the normal negative control. Determination of HBX protein-Paraffin sections of kidney tissue (3  $\mu$ m) were dewaxed and placed in water and stained with En Vision two-step method. EDTA (pH 8.0) repair solution was used for high-pressure repair antigen and rabbit polyclonal Anti-HBX antibody was added and incubated overnight at 4°. The corresponding secondary antibody labeled with

biotin was added, incubated at 4° for 20 min, washed with buffer for 2-3 times and stained with 3,3'-Diaminobenzidine (DAB), dehydrated, transparent and sealed. 20 visual fields were randomly selected for each image and 0-3+ were set from negative to the strongest. The negative control was set: phosphate buffer (PBS) instead of primary antibody. Tenofovir dipivoxil combined with Compound Yiganling Tablets was given 300 mg/time, once daily for 6 mo. Compound Yiganling Tablets 4 tablets/time, 3 times/d, continuous treatment for 6 mo. The basic treatment of all patients was collected and the patients were followed up for 6 mo. The collected data were input into the computer system and analyzed. Judgment of therapeutic effect<sup>[3]</sup>-Effectively, the urinary protein of patients was decreased by  $\geq 50\%$  compared with that before treatment, albumin (ALB) was increased and  $\geq 30$  g/L, alanine aminotransferase (ALT) and HBV-DNA were decreased by  $\geq 50\%$  compared with that before treatment, and the symptoms and signs were relieved. Failure to meet the above standards. Statistical package for social sciences (SPSS) 22.0 software was used. Measurement data were expressed as ( $\bar{x}\pm s$ ) and t test was used to analyze the difference between groups. Count data using frequency or percentage,  $\chi^2$  test analysis between groups differences;  $\alpha=0.05$ , Table 1. There was no significant difference in white blood cells (WBC), red blood cells (RBC), platelet count test (PLT), activated partial thromboplastin time (APTT), prothrombin time (PT), ALT, AST between the observation group and the control group ( $p>0.05$ ). See Table 2. The positive rate of HBX protein and the proportion of HBV-DNA  $>105$  IU/ml in the observation group were significantly higher than those in the control group ( $p<0.05$ ), while the proportion of HBX protein and HBV-DNA  $<103$  IU/ml in the observation group were significantly lower than those in the control group ( $p<0.05$ ). See Table 3. There was no significant difference in gender, age, body mass index and positive rate of HBX protein between the patients with and without Sanyang in the observation group ( $p>0.05$ ). The proportion of HBV-DNA  $>105$  IU/

**TABLE 1: COMPARISON OF GENDER AND AGE BETWEEN OBSERVATION GROUP AND CONTROL GROUP**

Group	cases	Male/ female	Age (y)	Body mass index (kg/m <sup>2</sup> )
observation group	78	48/30	45.50 $\pm$ 9.97	22.45 $\pm$ 3.36
control group	70	41/29	43.84 $\pm$ 10.15	21.97 $\pm$ 2.95
t		0.135	1.003	0.919
p		0.713	0.318	0.360

**TABLE 2: COMPARISON OF BLOOD ROUTINE AND BIOCHEMICAL INDEXES BETWEEN THE OBSERVATION GROUP AND THE CONTROL GROUP**

Index	observation group (n=78)	control group (n=70)	t/ $\chi^2$	P
WBC ( $\times 10^9/L$ )	6.45 $\pm$ 1.12	6.80 $\pm$ 1.21	-1.827	0.070
RBC ( $\times 10^{12}/L$ )	4.42 $\pm$ 0.97	4.55 $\pm$ 0.91	-0.838	0.403
PLT ( $\times 10^9/L$ )	199.78 $\pm$ 33.87	202.45 $\pm$ 36.68	-0.460	0.646
APTT (s)	32.10 $\pm$ 6.10	33.15 $\pm$ 6.87	-0.985	0.326
PT (s)	11.65 $\pm$ 3.31	11.42 $\pm$ 3.28	0.424	0.672
ALT (U/L)	32.29 $\pm$ 9.97	30.87 $\pm$ 8.11	0.944	0.347
AST (U/L)	36.61 $\pm$ 10.05	34.48 $\pm$ 11.17	1.221	0.224
GGT (U/L)	48.84 $\pm$ 16.67	45.56 $\pm$ 17.19	1.178	0.241
ALB (g/L)	21.45 $\pm$ 2.15	20.87 $\pm$ 2.25	1.603	0.111
TBIL (mol/L)	9.41 $\pm$ 2.03	9.69 $\pm$ 1.94	-0.855	0.394
DBIL (mol/L)	2.60 $\pm$ 0.95	2.71 $\pm$ 0.96	-0.700	0.485
BUN (mol/L)	6.61 $\pm$ 1.12	6.40 $\pm$ 1.09	1.153	0.251
Scr (mol/L)	76.65 $\pm$ 10.15	80.15 $\pm$ 12.21	-1.903	0.059
24h urinary protein quantification (g)	4.82 $\pm$ 0.99	4.76 $\pm$ 1.00	0.366	0.715

**TABLE 3: COMPARISON OF HBV MARKERS, HBX AND HBV-DNA BETWEEN OBSERVATION GROUP AND CONTROL GROUP**

Index	observation group (n=78)	control group (n=70)	t/ $\chi^2$	p
Big three positive	48 (61.54)	18 (25.71)	19.162	0.000
Small three positive	25 (32.05)	50 (71.43)	22.886	0.000
other	5 (6.41)	2 (2.86)	0.395	0.529
HBx protein positive rate (%)	70 (89.74)	45 (64.29)	13.800	0.000
HBV-DNA quantification (IU/ml)				
<10 <sup>3</sup>	9 (11.54)	29 (41.43)	17.272	0.000
10 <sup>3</sup> ~10 <sup>5</sup>	28 (35.90)	30 (42.86)	0.750	0.387
>10 <sup>5</sup>	41 (52.56)	11 (15.71)	21.981	0.000

Note: Big three positive: HBsAg, HBeAg, HBcAb positive; small three positive: HBsAg, HBeAb, HBcAb positive.

**TABLE 4: COMPARISON OF HBX AND HBV-DNA IN PATIENTS WITH BIG THREE POSITIVE AND NON BIG THREE POSITIVE IN OBSERVATION GROUP**

Index	Big three positive (n=48)	Non big three positive (n=30)	t/ $\chi^2$	P
Sex				
Male	30 (62.50)	18 (60.00)	0.049	0.825
Female	18 (37.50)	12 (40.00)		
Age (y)	46.20 $\pm$ 9.72	44.38 $\pm$ 10.15	0.791	0.431
body mass index (kg/m <sup>2</sup> )	22.29 $\pm$ 2.81	22.71 $\pm$ 3.00	-0.626	0.533
HBX Protein positive rate (%)	45 (93.75)	25 (83.33)	1.192	0.275
HBV-DNA quantification				
<10 <sup>3</sup>	3 (6.25)	7 (23.33)	3.413	0.065
10 <sup>3</sup> ~10 <sup>5</sup>	15 (31.25)	12 (40.00)	0.625	0.429
>10 <sup>5</sup>	30 (62.50)	11 (36.67)	4.941	0.026

ml in the observation group was significantly higher than that in the non-three positive patients ( $p < 0.05$ ). See Table 4. The proportion of HBV-DNA quantification >105 IU/ml in the observation group was 76.00 %, which was significantly higher than that in the patients with effective treatment ( $p < 0.05$ ). There was no

significant difference in gender, age, body mass index, hepatitis B marker phenotype and positive rate of HBX protein between ineffective and effective patients in the observation group ( $p > 0.05$ ). See Table 5. Hepatitis B virus associated nephritis is a kind of immune complex renal disease caused by hepatitis B virus directly or

**TABLE 5: COMPARISON OF HBX AND HBV-DNA BETWEEN EFFECTIVE AND INEFFECTIVE PATIENTS IN THE OBSERVATION GROUP**

Index	Effective (n=53)	Ineffective (n=25)	t/ $\chi^2$	p
Sex				
Male	31 (58.49)	17 (68.00)	0.649	0.420
Female	22 (41.51)	8 (32.00)		
Age (y)	46.03±8.12	44.65±9.12	0.673	0.503
body mass index (kg/m <sup>2</sup> )	22.17±3.03	22.90±3.16	-0.980	0.330
Hepatitis B marker phenotype				
big three positive	33 (62.26)	16 (64.00)	0.022	0.882
Non big three positive	20 (37.74)	9 (36.00)		
HBX Protein positive rate (%)	46 (86.79)	24 (96.00)	0.724	0.395
HBV-DNA quantification				
<10 <sup>3</sup>	9 (16.98)	1 (4.00)	1.531	0.216
10 <sup>3</sup> ~10 <sup>5</sup>	22 (41.51)	5 (20.00)	3.472	0.062
>10 <sup>5</sup>	22 (41.51)	19 (76.00)	8.104	0.004

indirectly. Its diagnosis requires renal biopsy and serum immunology. Mild hematuria, proteinuria and nephrotic syndrome are the main clinical manifestations of most patients with hepatitis B virus associated nephritis<sup>[4]</sup>. At present, the specific mechanism of this disease is not clear. Some studies believe that the accumulation of HBV antigen-antibody complexes in the glomerular site causes the immune loss of the body, which leads to the occurrence and development of this disease. It mainly exists in two forms: circulating immune complexes and *in situ* immune complexes. Some scholars believe that the virus directly infects kidney cells and causes kidney damage. The pantropism of HBV can directly infect kidneys, destroy kidney cells and cause death<sup>[5]</sup>. HBV virus can activate the apoptosis pathway *in vivo* to induce apoptosis, change the expression of podocyte surface markers, activate cellular immunity, promote the synthesis and release of cytokines, and cause local immune response<sup>[6]</sup>. At present, the coexistence of HBV infection and immune-mediated inflammation leads to difficult treatment. There is no unified and clear treatment plan at present. The main principle of clinical treatment is to reduce urinary protein, protect liver and kidney function, slow down the progress of the disease and prevent the recurrence of the disease<sup>[7]</sup>. Studies have shown that inhibition of HBV-DNA replication and effective clearance of HBe Ag can reduce proteinuria, even negative and improve renal function in patients with HBV-related nephropathy<sup>[8]</sup>. This study analyzed the expression of hepatitis B virus X (HBX) protein and hepatitis B virus DNA (HBV-DNA) in hepatitis B virus associated nephritis and its role in clinical treatment evaluation. HBX protein is one of the basic viral proteins in the viral infection cycle *in vivo* and is one of

the indispensable proteins for virus replication *in vivo*. Animal experiments showed that the replication of HBV in liver cells in mice with cancer was dependent on the expression of X protein. HBX protein had a wide range of effects on the proliferation of glomerular mesangial cells, the apoptosis of podocytes and renal tubular epithelial cells<sup>[9-10]</sup>. Some scholars have found that HBX protein is closely related to the occurrence and development of renal diseases. The positive rate of HBV-DNA expression in renal tissues with HBV serological positive detection by *in situ* hybridization is 88.9 % and the distribution of cytoplasm in renal tubular epithelial cells is consistent with that of HBVAg positive particles in tissues<sup>[11-12]</sup>. HBV gene is a circular DNA molecule with incomplete double-stranded, highly conserved sequence is the basic viral protein in the virus infection cycle *in vivo*, mainly located in the host cytoplasm, a small part in the nucleus<sup>[13]</sup>. Quantitative detection of HBV-DNA can reflect the replication of HBV virus and the level of HBV in serum. In this study, the proportion of HBV-DNA >105 IU/ml in patients with triple positive was significantly higher than that in patients with non-triple positive and after active treatment, HBV-DNA was significantly decreased, indicating that the status of viral replication and viral load after HBV infection are related to the occurrence of this disease, consistent with previous studies<sup>[14-15]</sup>. This also suggests that the clinical HBe Ag positive, HBV-DNA quantitative higher patients, should actively anti-HBV treatment, maximum inhibition of viral replication. The results showed that the positive rates of HBX protein and HBV-DNA >105 IU/ml in the observation group were significantly higher than those in the control group, while the positive rates of HBX protein and HBV-DNA <103 IU/ml in the

observation group were significantly lower than those in the control group. It is suggested that the positive rate of 'three positive' and HBX protein and the proportion of HBV-DNA >105 IU/ml in patients with hepatitis B virus associated nephritis are significantly increased, suggesting that viral replication is more significant. The proportion of HBV-DNA >105 IU/ml in the observation group was significantly higher than that in the non-three positive patients, suggesting that the viral load in patients with hepatitis B virus-associated nephritis was higher. Through active and effective treatment, the proportion of patients with HBV-DNA >105 IU/ml in the observation group was 76 %, through active and effective treatment, the proportion of patients with HBV-DNA quantification >105 IU/ml in the observation group was 76.00 %, which was significantly higher than that of patients with effective treatment, suggesting that the level of HBV-DNA was related to the clinical therapeutic effect. The higher the proportion of patients with HBV-DNA quantification >105 IU/ml, the worse the therapeutic effect. This study analyzed the expression of HBX protein and HBV-DNA quantification in patients with hepatitis B virus-associated nephritis, and confirmed that the level of HBV-DNA had a certain relationship with clinical efficacy, which provided a certain basis for clinical rational evaluation of clinical efficacy. However, the number of patients included in this study was small and the specificity and sensitivity analysis was not carried out. Therefore, it is necessary to further expand the sample size and prolong the follow-up time. In summary, the levels of HBX protein and HBV-DNA in patients with hepatitis B virus-associated glomerulonephritis were relatively high and the level of HBV-DNA was related to Dasanyang and therapeutic efficacy, while HBX protein was not significantly correlated.

### Conflict of interests:

The authors declared no conflicts of interest.

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