



## Status of Hepatitis B Virus Surface Antigen (HBsAg) Deposition in Renal Tissue among Glomerulonephritis Patients

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### Abstract

**Background:** Hepatitis B virus (HBV) related glomerulonephritis (GN) may be found in HBV seropositive as well as seronegative patients. HBV may present in the renal tissue of such patients. **Objective:** The purpose of the present study was to assess status of HBsAg deposition in renal tissue among glomerulonephritis patients. **Methodology:** This cross-sectional study was conducted in the Department of Nephrology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh during the period of July 2015 to June 2016. All diagnosed GN patients both HBV seropositive and seronegative aged more than 18 years of both sexes admitted in the department of Nephrology BSMMU were taken as the study population. Serological tests both HBsAg and anti-HBc (total) were done to identify the HBV seropositive and seronegative individuals by ELISA technique and Chemiluminescence Method respectively. Renal biopsy was done percutaneously through a posterior approach. Immunohistochemistry and one core were preserved in normal saline for direct immunofluorescence (DIF). Both test tubes were sent to find out histological features and presence of HBsAg in renal biopsy tissue in Pathology Department of Bangabandhu Sheikh Mujib Medical University, Dhaka. **Results:** HBsAg antigen in renal tissue was found in 2 patients among 7 patients who were seropositive for both HBsAg and Anti-HBc antibody (total), 2 patients among 8 patients who were HBsAg seronegative but Anti-HBc (total) seropositive, 2 patients among 38 patients who were seronegative for both HBsAg and Anti-HBc (total). There were no patients in this study who was HBsAg seropositive but Anti HBc (total) seronegative. Total 11.3% (6 patients) of renal biopsy specimens were found to have HBsAg deposits which included 3 cases of Membranoproliferative GN and one of each of Membranous nephropathy, Mesangial proliferative GN and IgA nephropathy. **Conclusion:** The high rate of HBsAg deposits has been found in renal tissue among GN patients.

**Keywords:** HBsAg; Anti-HBc (total); Renal biopsy; Glomerulonephritis

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### Introduction

Hepatitis B virus (HBV) infection is a worldwide epidemic and is particularly prevalent in developing

countries such as those in Southeast Asia and Africa. In addition to liver damage, HBV infection causes manifestations in other organs, which is increasingly recognized as major pathogenesis of HBV-related morbidity and mortality. Among the extra-hepatic manifestations related to HBV infection, HBV-related nephritis is a major manifestation by HBV infection<sup>1</sup>. Different pathological types of glomerular lesions have been described in association with HBV infection,

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including pathological patterns such as Membranoproliferative Glomerulonephritis (MPGN), Mesangialproliferative glomerulonephritis (MesPGN), and Membranous glomerulonephritis (MN). However, among these histological types, MN has been reported as the commonest pathological type of HBV-GN in Hong Kong and South Africa, compared to IgA nephropathy (IgAN) followed by MN in Thailand and MN and MPGN in Japanese adults<sup>2</sup>.

Approximately one-third of the world's population has serological evidence of past or present infection with HBV and it is estimated that 350 million people are chronically infected, making it one of the most common human pathogens<sup>3,4</sup>. The spectrum of disease and natural history of chronic HBV infection are diverse and variable, ranging from an inactive carrier state to progressive hepatic and extrahepatic (Renal) manifestations but it is not possible to predict which patient with HBV infection are more likely to develop kidney disease<sup>5</sup>. Pathogenesis of HBV related nephritis is mediated by subendothelial and mesangial deposition of immune complexes<sup>6</sup>.

The diagnosis of HBV-related glomerulonephritis is based on established criteria. Patients with serum and renal tissue HBV antigens with symptoms and signs of glomerulonephritis and without other secondary diseases were diagnosed as HBV-related glomerulonephritis<sup>7</sup>. In fact, HBV infections are sometimes occult. These patients are characterized by the presence of HBV infection with undetectable HBsAg antigen in serum, whereas the viral DNA may be present in the blood or tissues, or the tissues may be positive for viral antigens. Occult HBV infections have often been neglected. However, they can also cause immune complex deposition in tissues. Hence an occult HBV infection may cause HBV-associated glomerulonephritis as well<sup>8</sup>.

Hepatitis-B-associated glomerulonephritis (HBGN) is a distinct entity occurring frequently in hepatitis-B-prevalent areas of the world. The disease affects both adults and children who are chronic hepatitis-B-virus (HBV) carriers with or without a history of overt liver disease. The diagnosis is established by serologic evidence of HBV antigens/antibodies, presence of immune complex glomerulonephritis, immunohistochemical localization of 1 or more HBV antigens, and pertinent clinical history, when available<sup>9</sup>.

In this study, detection of HBsAg antigen was performed in the renal tissues of both seropositive and seronegative patients with glomerulonephritis. The

results may provide clues to the underlying aetiology and may help to formulate treatment plan for the management of glomerulonephritis patients.

## Methodology

**Study Population and Settings:** This cross-sectional study was carried out in the Department of Nephrology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from January 2015 to September 2016. All diagnosed GN patients with both HBV seropositive and seronegative aged more than 18 years of both sexes who were admitted in the Department of Nephrology of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh were taken as the study population. GN patients with contracted kidneys, Patients who refuse biopsy, Patients with contraindications to renal biopsy, Patients who are unwilling to participate in the study were excluded. Serological tests both HBsAg and anti-HBc (total) were done to identify the HBV seropositive and seronegative individuals by ELISA technique and Chemiluminescence Method respectively. Then after proper counseling and consent; renal biopsy was done percutaneously through a posterior approach. One core of tissue was preserved in formalin in a test tube for light microscopic study and immunohistochemistry and one core was preserved in normal saline for direct immunofluorescence (DIF). Both test tubes were sent to find out histological features and presence of HBsAg in renal biopsy tissue in Pathology Department of Bangabandhu Sheikh Mujib Medical University, Dhaka. Tissue for light microscopic examination was fixed in 10% formalin, processed routinely for paraffin section. From paraffin embedded material 5 micron thick tissue sections were stained by hematoxyllin and eosin, periodic acid Schiff's (PAS) methods. Ethical approval had been taken from the local Institutional Review Board of of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The reference number of IRB approval was BSMMU/2015/11022, Date: 30/08/2015

## Immunofluorescence Microscopic Examination:

Tissue kept in normal saline was used for direct immunofluorescence study. Specimen were placed on block holder and rapidly frozen and embedded in O.C.T compound (Thermoshandon, Pittsburgh PA, and USA). Cryostat sections were cut at -20°C cooled chamber at 4-5 micron thickness and was then collected on glass slides. The sections were air dried and kept in deep freeze at -20°C until staining.

Staining were done by incubating the sections with FITC (Fluorescein isothiocyanate) conjugated rabbit antisera against human IgG, IgM, IgA, C3 and fibrinogen. The sections were then examined under fluorescence microscope. During microscopy photomicrographs were taken for each positive case.

**Immunohistochemistry Examination:** In this study immunohistochemical stain was done to detect viral antigens in the kidney tissue. Streptavidin-Biotin system for immuno-peroxidase stain was used on deparaffinized tissue sections. Primary antibodies used were monoclonal mouse anti-hepatitis B virus surface antigen (Thermo Scientific USA). The streptavidin-peroxidase kit was used as a secondary antibody. Staining was visualized using 3,3'-diaminobenzidine (DAKO, Denmark).

**Statistical Analysis:** The result was presented as mean±SD or median, according to normality characteristics of each variable with 5% ( $p < 0.05$ ) significance level. To compare parametric variables paired t-test was used. Data was analyzed using Statistical Package for Social Science (SPSS) v 22.0 (Chicago, IL) software.

## Results

A total number of 53 patients were recruited for this study. The mean age was  $34.5 \pm 12.33$  years with minimum 18 and maximum 60 years. Most of the patients were in the 18 to 29 years of age group which was 21(39.6%) cases. About 25(47.2%) patients were male and 28(52.8%) were female. Sex distribution was thus approximately equal (Table 1).

Table 1: Distribution of the Study Patients According to Age Group (n=53)

Variables	Frequency	Percent
<b>Age Group</b>		
18 to 29 Years	21	39.6
30 to 39 Years	15	28.3
40 to 49 Years	7	13.2
50 to 60 Years	10	18.9
Mean±SD (Range)	34.5±12.33 (18-60) Years	
<b>Gender</b>		
Male	25	47.2
Female	28	52.8

Among 7 seropositive cases HBsAg antigen was found in renal tissue only 2(28.6%) and was absent in 5(71.4%) cases. However among 46 HBsAg seronegative cases HBsAg antigen was present in renal

tissue in 4(8.7%) and absent in 42(91.3%) cases (Table 2).

Among 15 anti-HBc (total) seropositive cases HBsAg antigen was found in renal tissue only 4(26.7%) cases and was absent in 11(73.3%) cases. However, among 38 Anti-HBc (total) seronegative cases HBsAg antigen was present in renal tissue in 2(5.3%) and absent in 36(94.7%) cases (Table 3).

Table 2: Presence of HBsAg Deposition in Renal Tissue in HBsAg Seropositive and Seronegative Cases (n=53)

HBsAg Sero-Status	HBsAg Deposition in renal tissue	
	Present	Absent
Seropositive	2(28.6%)	5(71.4%)
Seronegative	4(8.7%)	42(91.3%)
<b>Total 3</b>	<b>6(11.3%)</b>	<b>47(88.7%)</b>

Table 3: Presence of HBsAg Deposition in Renal Tissue in Anti-HBc (total) Seropositive and Seronegative Cases (n=53)

Anti-HBc (Total) Sero-Status	HBsAg Deposition in Renal Tissue	
	Present	Absent
Seropositive	4(26.7%)	11(73.3%)
Seronegative	2(5.3%)	36(94.7%)
<b>Total</b>	<b>6(11.3%)</b>	<b>47(88.7%)</b>

Among 6 cases with HBsAg antigen deposition in renal tissue, 1(16.7%) case, 3(50.0%) case, 1(16.7%) case and 1(16.7%) case were MN, MPGN, MesPGN and IgAN respectively (Table 4).

Table 4: Histological Types of GN Patients with HBsAg Deposition in Renal Tissue (n=6)

Histological Types of GN	Frequency	Percent
MN	1	16.7
MPGN	3	50.0
MesPGN	1	16.7
FSGS	0	0.0
IgAN	1	16.7
LN	0	0.0
<b>Total</b>	<b>6</b>	<b>100.0</b>

Among 53 patients in the sample, 2 cases had HBsAg in both serum and renal tissue as well as Anti-HBc (Total) seropositivity. About 2 cases had HBsAg deposits in renal tissue but were HBsAg seronegative and anti-HBc (total) seropositive. About 5 patients had both HBsAg and Anti HBc (total) seropositivity but no HBsAg deposit in renal tissue, there were 6 patients who were HBsAg seronegative and Anti-HBc seropositive but no HBsAg deposit in renal tissue was found. 2 cases had HBsAg deposits in renal tissue but both HBsAg and Anti-HBc (Total) were seronegative.

The rest 36 patients had neither HBsAg deposit in renal tissue nor HBsAg (Total) seropositivity (Table 5).

Table 5: Presence of HBsAg Deposition in Renal Tissue in HBsAg and Anti-HBc (total) Seropositive and Seronegative Cases (n=53)

Sero -status of HBV Antigen and Antibodies	HBsAg Deposition in Renal Tissue	
	Present	Absent
HBsAg Seropositive + Anti HBc (total) Seropositive	2(28.6%)	5(71.4%)
HBsAg Seropositive + Anti HBc (total) Seronegative	0(0.0%)	0(0.0%)
HBsAg seronegative + Anti HBc (total) Seropositive	2(25%)	6(75%)
HBsAg seronegative + Anti HBc (total) Seronegative	2(5.3%)	36(94.7%)
<b>Total</b>	<b>6(11.3%)</b>	<b>47(88.7%)</b>

### Discussion

This study was conducted to estimate the frequency of HBsAg deposition in renal tissue of patients with glomerulonephritis (GN). Twenty-one (39.6%) among 53 patients were in the 18 to 29 years of age group. Mean age was 34.5±12.33 years, minimum 18 years and maximum 60 years. 25(47.2%) patients were male and 28(52.8%) were female.

In this study among 53 study subjects, 7 (13.2%) cases were HBsAg seropositive and 46(86.8%) cases were HBsAg seronegative. Among the 7 seropositive cases, HBsAg antigen deposition in renal tissue was present in 2 (28.6%) and absent from 5(71.4%) cases. Among the 46 HBsAg seronegative cases, HBsAg antigen deposition in renal tissue was present in 4(8.7%) and absent from 42(91.3%) cases. Among 53 study subjects 15(28.3%) cases were Anti-HBc (total) seropositive and 38(71.7%) cases were anti-HBc (total) seronegative. Among the 15 Anti-HBc (total) seropositive cases, HBsAg antigen deposition in renal tissue was present in 4(26.7%) cases and absent from 11(73.3%) cases. Among the 38 Anti-HBc (total) seronegative cases, HBsAg antigen deposition in renal tissue was present in 2(5.3%) cases and absent from 36(94.7%) cases.

Kong et al<sup>8</sup> found HBsAg antigen present in immunohistochemical staining in 3(0.6%) out of 500 renal biopsy cases. Among 3 patients with HBsAg antigen in renal tissue, 2 cases had Anti-HBc (total) positive in serum and 1 case did not. However, HBsAg sero-status was negative in all 500 patients in that study.<sup>8</sup> In this study among 7 HBsAg seropositive cases, histological types of GN were MN, MPGN, FSGS and LN and HBsAg antigen deposition was

found in 2(28.6%) cases. Among 15 Anti-HBc (Total) seropositive cases, histological types of GN were MN, MPGN, MesPGN, FSGS, IgAN, LN and HBsAg antigen deposition was found in 4(26.7%) cases. In this study 25(47.16%) patients presented with nephrotic syndrome where the most common histological types of GN were MesPGN 7(28.0%) cases and MN 5(20.6%) cases. Nephritic syndrome was found in 28(52.8%) patients out of which LN was established in 10(36%) cases, MesPGN in 8(29%) patients and MPGN in 7(25.0%) cases.

In this study, among the 7 HBsAg seropositive cases, 2 patients had HBsAg antigen present in renal tissue (1 MN, 1 MPGN). Among 46 HBsAg seronegative cases, 4 patients had HBsAg antigen present in renal tissue (2 MPGN, 1 MesPGN and 1 IgAN). Among 15 Anti-HBc (total) seropositive cases, 4 patients had HBsAg antigen present in renal tissue (1 MN, 2 MPGN, 1 IgAN). Among 38 Anti-HBc (Total) seronegative cases, 2 patients had HBsAg deposition in renal tissue (1 MPGN, 1 MesPGN). Amarapurakar et al.<sup>10</sup> conducted a study including 28 patients (10 HBsAg seropositive and 18 seronegative), all 10 HBsAg seropositive patients showed HBsAg deposits in renal tissue (Histology: MN in 4 patients and proliferative GN in 6 patients). Only 8 patients had HBsAg present (5 patients proliferative GN, 3 post-transplant rejection) in renal tissue among the other 18 seronegative patients with GN<sup>10</sup>. Among the 28 patients studied by Amarapurakar et al<sup>10</sup> 20 were Anti-HBc (total) seropositive cases and among them, 14 patients were found to have HBsAg antigen deposition in renal tissue. Meanwhile among the 8 Anti-HBc (total) seronegative cases and among them 4 patients were found HBsAg antigen deposition in renal tissue<sup>10</sup>.

This current study showed different histological types of GN patients had deposition of HBsAg in renal tissue. Among the 6 cases with HBsAg antigen in the renal tissue the following diagnoses were established: MPGN in 3(50.0%) cases, MN in 1(16.7%) cases and MesPGN in 1(16.7%) cases and IgAN 1(16.7%). Amarapurakar et al<sup>10</sup> found HBsAg antigen in both glomerulus and tubular epithelium which resemble the findings of our study.

### Conclusion

The endemicity of HBV nephropathy in our patients as hinted by the frequency of HBsAg deposition in renal biopsy specimens of GN patients is not insignificant. Not all patients who were seropositive for HBsAg and



anti-HBc (total) displayed HBsAg deposition in renal tissue. Moreover, even those patients who were seronegative for both HBsAg and Anti-HBc (total) were also found to have HBsAg antigen deposition in the renal tissue. Therefore all cases of GN should undergo renal biopsy and immunohistochemistry for diagnosis of HBV related GN irrespective of their HBsAg and/or anti-HBc (total) seropositivity.

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None

#### Conflict Of Interest

The authors have no conflicts of interest to disclose

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#### Authors' contributions

GM Hafizur Rahman conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Muhammad Rafiqul Alam, Hossain Muhammad Mustafijur Rahman contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Md. Abdullah Yusuf, Al Asma Ul Taslima, Faruque Ahmed involved in the manuscript review and editing. All authors read and approved the final manuscript.

#### Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

#### Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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