

## Original Article

## Relationship of HBsAg and Kidney Biopsy Marker with HBV Related Glomerulonephropathy

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## Abstract

Hepatitis B virus infection is a major public health problem worldwide and it causes not only hepatic diseases but also extra hepatic manifestations particularly HBV-associated Glomerulonephritis (GN). HBsAg has been observed in the glomeruli of some patients with glomerulonephritis. HBV related glomerulonephritis may be found in HBV seropositive as well as seronegative patients. HBV may present in the renal tissue of such patients. In most cases detection of HBsAg in the renal tissue by renal biopsy and immunohistochemistry can establish the etiology. To find out the relationship of HBsAg and Kidney biopsy marker with HBV related glomerulonephropathy, this cross sectional study was done in the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), during the period of July 2015 to June 2016. A total number of 53 cases who fulfilled the inclusion and exclusion criteria were selected as sample. Samples were selected by purposive sampling technique. HBsAg antigen in renal tissue was found

in 2 patients among 7 patients who were seropositive for both HBsAg and Anti HBc(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 Membrano- proliferative GN and one of each of Membranous nephropathy, Mesangial proliferative GN and IgA nephropathy. The high rate of HBsAg deposits found in renal tissue indicates that detection of HBsAg deposition should be done for all histological varieties of GN. Antiviral therapy may be used to stop progression of HBV associated nephropathy.

**Keywords:** HBsAg, Anti HBc (total), Renal Biopsy, Glomerulonephritis.

## 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 the liver damage, HBV infection causes manifestations in other organs, which is increasingly recognized as a 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, including pathological patterns such as Membrano- proliferative Glomerulonephritis (MPGN), Mesangial- proliferative 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

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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 an immune complex glomerulonephritis, immunohistochemical localization of 1 or more HBV antigens, and pertinent clinical history, when available.<sup>9</sup>

In this study we will detect HBsAg antigen in the renal tissues (obtained by Biopsy) of both seropositive and seronegative patients with GN. The results may provide clue to the underlying aetiology and may help to formulate treatment plan in the management of GN patients.

## MATERIALS AND METHODS

This was a cross sectional study carried out in the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from January 2015 to September 2016. A total number of 53 cases who

fulfilled the inclusion and exclusion criteria were selected as sample. Samples were selected by purposive sampling technique.

### Selection of Patients:

#### • Inclusion Criteria

- o Patients diagnosed as GN (both HBV seropositive and seronegative) admitted in the department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka.
- o Age:  $\geq$  18 years
- o Sex: Both Sexes
- o Patients who gave informed written consent.

#### • Exclusion Criteria

- o GN patients with contracted kidneys.
- o Patients with contraindications to renal biopsy.
- o Patients aged below 18 years.
- o Patients who are unwilling to participate in the study.
- o Patients with pregnancy.

### Study procedure:

Serological test 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.

### Immunofluorescence microscopy

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  $-20^{\circ}\text{C}$  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

In this study immunohistochemical stain was done to detect viral antigens in the kidney tissue. Streptavidine-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).

### Data collection, processing and analysis:

Relevant data were collected by using a preformed data sheet. All other required data was collected from history sheet, clinical examination and investigation papers. Renal biopsy as well as immunohistochemistry report was recorded in the data sheet.

Statistical analysis of the study was done by computer software device as the Statistical Package for Social Science (SPSS), version-22.0 (Chicago, IL) software. The result was presented in tables, figures and diagrams. Quantitative variables were expressed as mean±SD and significance was estimated with student's unpaired t-test while qualitative variables were expressed as frequency or percentage and their significance was assessed using Chi-square test. 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.

## RESULTS

This cross sectional study was conducted among 53 study subjects with Patients diagnosed as GN (both HBV seropositive and seronegative). Data were analyzed with SPSS software using appropriate statistical method and were presented in tables in this chapter.

Table-I shows MN and FSGS patients presented with nephrotic syndrome and the rest presented with either nephrotic or nephritic syndrome.

**Table-I: Clinical presentations in various histological types of GN (n=53)**

Histological types of GN	Clinical presentation		P value
	Nephrotic syndrome No. (%) (n=25)	Nephritic syndrome No. (%) (n=28)	
MN	5(20.6%)	0(0.0%)	0.005 <sup>s</sup>
MPGN	6(24.8%)	7(25.0%)	
MesPGN	7(28.0%)	8(28.6%)	
FSGS	4(16.0%)	0(0.0%)	
IgAN	2(8.0%)	3(10.7%)	
LN	1(4.0%)	10(35.7%)	
Total	25(100.0%)	28(100.0%)	

Table-II shows 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-II: Presence of HBsAg deposition in renal tissue in HBsAg seropositive and seronegative cases (n=53)**

HBsAg sero-status	n	HBsAg Deposition in renal tissue		P value
		Present No. (%)	Absent No. (%)	
HBsAg seropositive	7	2(28.6%)	5(71.4%)	0.174 <sup>ns</sup>
HBsAg seronegative	46	4(8.7%)	42(91.3%)	
Total	53	6(11.3%)	47(88.7%)	

Table-III shows that among 15 anti-HBc (total) seropositive cases HBsAg antigen was found in renal tissue in 4(26.7%) 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-III: Presence of HBsAg deposition in renal tissue in Anti-HBc (total) seropositive and seronegative cases (n=53)**

Anti-HBc (total) sero-status	n	HBsAg Deposition in renal tissue		P value
		Present No. (%)	Absent No. (%)	
Anti HBc (total) seropositive	15	4(26.7%)	11(73.3%)	0.026s
Anti HBc (total) seronegative	38	2(5.3%)	36(94.7%)	
Total	53	6(11.3%)	47(88.7%)	

Table –VII shows that among 53 total patients in the sample, 2 cases had HBsAg in both serum and renal tissue as well as Anti-HBc (total) seropositivity. 2 cases had HBsAg deposits in renal tissue but were HBsAg seronegative and anti-HBc (total) seropositive. 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 were 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) soropositivity.

**Table –V: 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	No (n)	HBsAg deposition in renal tissue	
		Present No (%)	Absent No (%)
HBsAg Seropositive + Anti HBc (total) seropositive	7	2(28.6%)	5(71.4%)
HBsAg Seropositive + Anti HBc (total) seronegative	0	0(0.0%)	0(0.0%)
HBsAg seronegative + Anti HBc (total) seropositive	8	2(25%)	6(75%)
HBsAg seronegative + Anti HBc (total) seronegative	38	2(5.3%)	36(94.7%)
Total	53	6 (11.3%)	47(88.7%)

**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 18-29 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

Table-IV shows that among 6 cases with HBsAg antigen deposition in renal tissue, 1(16.7%), 3(50.0%), 1(16.7%) and 1(16.7%) were MN, MPGN, MesPGN and IgAN respectively.

**Table-VI: Distribution of histological types of GN patients with HBsAg deposition in renal tissue (n=6)**

Histological types of GN	HBsAg present in renal tissue	
	No	Percentage (%)
MN	1	16.7
MPGN	3	50.0
MesPGN	1	16.7
FSGS	0	0.0
IgAN	1	16.0
LN	0	0.0
Total	6	100.0

(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 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. (2013) found HBsAg antigen present in immunohistochemical staining in 3(0.6%) out of 500 renal biopsy cases.<sup>8</sup> Among 3 patients with HBsAg antigen in renal tissue, 2 had Anti-HBc (total) positive in serum and 1 did not. However HBsAg serostatus was negative in all 500 patients in that study.

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. (2015) 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. Among the 28 patients studied by Amarapurakar 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>

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.

(2015) found HBsAg antigen in both glomerulus and tubular epithelium which resemble the findings of our study.<sup>10</sup>

## CONCLUSIONS

This study showed different histological types of GN patients had deposition of HBsAg in renal tissue. All patients with seropositive for HBsAg and anti-HBc (total) did not displayed HBsAg deposition in renal tissue and patients with seronegative for both HBsAg and Anti-HBc (total) were also found the HBsAg antigen deposition in the renal tissue. 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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