

**DETECTION OF HBsAg IN RENAL TISSUE OF PATIENTS WITH  
GLOMERULONEPHRITIS**

**Dr. G. M. Hafizur Rahman<sup>\*1</sup>, Professor (Dr.) Muhammad Rafiqul Alam<sup>2</sup>, Dr. Hossain Muhammad Mustafijur Rahman<sup>3</sup>, Dr. Al Asma Ul Taslima<sup>4</sup>, Dr. Tanvir Rahman<sup>5</sup>, Dr. Faruque Ahmed<sup>6</sup>, Dr. Nahid Afrin<sup>7</sup>, Dr. Md Dilder Hossain Badal<sup>8</sup> and Dr. Golam Mahbub Sikder<sup>9</sup>**

<sup>1</sup>Assistant Professor, Department of Nephrology, Dhaka Medical College, Dhaka, Bangladesh.

<sup>2</sup>Professor and Ex-Chairman, Department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

<sup>3</sup>Assistant Professor, Department of Nephrology, Dhaka Medical College, Dhaka, Bangladesh.

<sup>4</sup>Junior Consultant (Radiology and Imaging), Dhaka Medical College Hospital, Dhaka, Bangladesh.

<sup>5</sup>Nephrologist, United Hospital Ltd, Dhaka, Bangladesh.

<sup>6</sup>Assistant Professor (Nephrology), Sheikh Russel Gastro Liver Institute and Hospital, Dhaka, Bangladesh.

<sup>7</sup>Nephrologist, National Institute of Kidney Diseases and Urology, Dhaka, Bangladesh.

<sup>8</sup>Nephrologist, Department of Nephrology, Dhaka Medical College Hospital, Dhaka, Bangladesh.

<sup>9</sup>Nephrologist, Department of Nephrology, Cumilla Medical College Hospital, Cumilla, Bangladesh.

**\*Corresponding Author: Dr. G. M. Hafizur Rahman**

Assistant Professor, Department of Nephrology, Dhaka Medical College, Dhaka, Bangladesh.

Article Received on 10/12/2020

Article Revised on 30/12/2020

Article Accepted on 20/01/2021

**ABSTRACT**

**Introduction:** Hepatitis B virus infection is a severe 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. **Objective:** To estimate the frequency of HBsAg deposition in renal tissue of patients with glomerulonephritis. **Method:** This cross sectional study was done in the Department of Nephrology, 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. **Results:** 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 Membranoproliferative GN and one of each of Membranous nephropathy, Mesangial proliferative GN and IgA nephropathy. **Conclusion:** 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. So that antiviral therapy can 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 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 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.

## METHODOLOGY

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. All diagnosed GN patients (both HBV seropositive and seronegative) aged more than 18 years of both sexes admitted in the department of Nephrology BSMMU was 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 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°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. 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).

## Data collection, processing and 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****Table-1: Distribution of the study patients according to age (n=53).**

Age group (years)	Frequency	Percentage
18-29	21	39.6
30-39	15	28.3
40-49	7	13.2
50-60	10	18.9
Total	53	100.0
Mean±SD Range	34.5±12.33 (18-60) yrs	

Table-1 shows Mean age was 34.5±12.33 years, minimum 18 and maximum 60 years. Most of the patients 21(39.6%) fell in the 18-29 year age group.

**Table-II: Distribution of the study patients according to sex (n=53).**

Sex	Frequency	Percentage (%)
Male	25	47.2
Female	28	52.8
Total	53	100

Table-II shows sex distribution, 25(47.2%) patients were male and 28(52.8%) were female. Sex distribution was thus approximately equal.

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

**Table-IV: 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-IV 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-V: 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%)	0.026 <sup>s</sup>
Anti HBc (total) seronegative	38	2(5.3%)	36(94.7%)	
Total	53	6(11.3%)	47(88.7%)	

Table-V shows Among 15 anti-HBc (total) seropositive cases HBsAg antigen was found in renal tissue only 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-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	16.7
LN	0	0.0
Total	6	100.0

Table-VI shows 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 VII: 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%)

Table -VII shows 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) seropositivity.

## 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 (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)<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 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)<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. Among the 28 patients studied by Amarapurakar<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.

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.* (2015)<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.

## REFERENCE

1. Tan Z, Fang J, Lu JH, Li WG. HBV serum and renal biopsy markers are associated with the clinicopathological characteristics of HBV-associated nephropathy. *International journal of clinical and experimental pathology*, 2014; 7(11): 8150-53.
2. Zhang L, Meng H, Han X, Han C, Sun C, Ye F, Jin X. The relationship between HBV serum markers and the clinicopathological characteristics of hepatitis B virus-associated glomerulonephritis (HBV-GN) in the northeastern chinese population. *Virology journal*, 2012 Dec; 9(1): 200-8.
3. McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology*, 2009; 49(5): 45.
4. European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *Journal of hepatology*, 2012; 57(1): 167.
5. Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. *Journal of Hepatology*, 2001; 34(4): 617-624
6. Waikhom R, Sarkar D, Patil K, Pandey R, Dasgupta S, Jadhav J, Abraham A. Non-IgA mesangioproliferative glomerulonephritis: a benign entity?. *Nephrology Dialysis Transplantation*, 2011 Nov 29; 27(6): 2322-7.
7. Chen L, Wu C, Fan X, Gao J, Yin H, Wang T, Wu J, Wen SW. Replication and infectivity of hepatitis B virus in HBV-related glomerulonephritis. *International Journal of Infectious Diseases*, 2009 May 1; 13(3): 394-8.
8. Kong D, Wu D, Wang T, Li T, Xu S, Chen F, Jin X, Lou G. Detection of viral antigens in renal tissue of glomerulonephritis patients without serological evidence of hepatitis B virus and hepatitis C virus infection. *International Journal of Infectious Diseases*, 2013 Jul 1; 17(7): e535-8.
9. Venkateshan VS, Lieberman K, Kim DU, Thung SN, Dikman S, D'agati V, Susin M, Valderrama E, Gauthier B, Prakash A. Hepatitis-B-associated glomerulonephritis: pathology, pathogenesis, and clinical course. *Medicine*, 1990 Jul; 69(4): 200-16.
10. Amarapurkar DN, Kirpalani AL, Amarapurkar AD. Role of hepatitis b in glomerulonephritis, *J of Ind Gastroenterology-Hepatology*, 2015; 1: 1-6.