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A renal biopsy study of hepatitis B virus-associated nephropathy in Korea

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A renal biopsy study of hepatitis B virus-associated nephropathy in Korea. The pathogenic role of the hepatitis B virus (HBV) infection for glomerulonephritis (GN) is not clear. The frequency of HBsAg has been studied in sera of 732 consecutive patients who have glomerular diseases by using radioimmunoassay. The frequency of HBs antigenemia was 11.9%, which was not different from that in the general population of South Korea. Of the 87 HBsAg seropositive patients with GN, 29 cases with membranoproliferative GN (MPGN) and eighteen with membranous nephropathy (MN) were diagnosed as having HBV-associated nephropathy. Eighty-seven and one-half percent of the adults with MPGN and 80% of the children with MN were HBsAg carriers. The morphologic findings and laboratory data in cases with HBV-associated MPGN and MN did not differ significantly from those observed in patients with MPGN and MN without circulating HBsAg. Yet mesangial deposits were more frequently noted in patients with HBV-associated MN when compared to others with idiopathic MN. Glomerular deposits of HBsAg were not detected using indirect immunofluorescence technique. Even though HBsAg was not demonstrable within the glomeruli, HBV infection seems to play an important role in the pathogenesis of MPGN in Korean adults and MN in children.

The geographical distribution of hepatitis B virus (HBV) infection varies considerably ranging from 0.1% in parts of Western Europe, North America and Australia to 20% or even higher in some populations of Asian, African and Western Pacific countries [1]. HBV infection has been involved in several forms of immune-related glomerulopathy, such as membranous nephropathy (MN) and membranoproliferative glomerulonephritis (MPGN). Since Combes et al [2] first reported the association of persistent Australia antigenemia and MN, many attempts have been made to define the morphological and clinical characteristics of HBV-associated nephropathy and to demonstrate antigenic material within the glomeruli of patients. Hepatitis B surface antigen (HBsAg) has been most often the only antigen studied in the serum as well as in the glomerular deposits. Although a number of authors [2–16] reported that HBsAg was stained by direct or indirect fluorescent antibody technique, the others [17–23] could not confirm the observation. Furthermore, the presence of HBsAg was demonstrated in the glomeruli of patients who had no detectable HBsAg in the serum [4, 8, 10, 12, 24, 25]. Such conflicting results made some researchers doubt the specificity of fluorescent staining for HBsAg within the deposits [10, 24, 26, 27]. In addition, the limited amounts of information, gained mostly from studies on children, have precluded characterization of the clinical and pathological features of HBV-associated nephropathy.

In South Korea where HBV infection is particularly prevalent, with a frequency of HBsAg carriers as high as 10 to 15% [28], the percentage of patients with both glomerular disease and circulating HBsAg is also high. In this paper, we attempt to examine the frequency of HBs antigenemia in patients with glomerular diseases and to describe morphological and clinical features of HBV-associated nephropathy.

Methods

Patients

A total of 732 consecutive patients with glomerular diseases was evaluated at the Department of Pathology, Seoul National University during the 30 month period ending in October 1987. Five hundred and sixty patients were adults and 172 were children. All of the specimens were from patients residing in South Korea referred from hospitals throughout the country. The circulating HBsAg and antibody (anti-HBs), hepatitis B e antigen (HBeAg) and antibody (anti-HBe), and anti-hepatitis B core antibody (anti-HBc) were screened by radioimmunoassay (RIA; kits AUSRIA II, AUSAB, ABBOTT-HBe, CORAB, Abbott Lab., Chicago, Illinois, USA). For each patient data were collected concerning age, sex, date of clinical onset, blood pressure, routine renal and liver function tests, plasma protein, antistreptolysin-o titer, LE cells, anti-DNA antibodies, antinuclear factor, complement C3, complement C4, complement hemolytic activity CH50, urinalysis and 24-hour urinary protein quantitation.

Pathological study

Renal biopsies, all of which contained at least six glomeruli, were processed for light, electron and immunofluorescent microscopy using standard methodologies previously described [29].

Light- and electron microscopy. Biopsy specimens for light microscopy were fixed in Zenker's fixative, embedded in

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Table 1. Renal histopathology and circulating hepatitis B surface antigen (HBsAg) in patients with glomerular lesions

Diagnosis	Number examined			Number of HBsAg carriers		
	Adults	Children	Total	Adults	Children	Total
Epithelial cell disease	92	48	140	3	3	6
Focal segmental glomerulosclerosis	48	22	70	3	3	6
Membranous nephropathy	49	10	59	10	8	18
Membranoproliferative GN	32	4	36	28	1	29
IgA nephropathy	192	22	214	15	1	16
Postinfectious GN	21	22	43	2	2	4
Mild focal nonspecific GN	41	20	61	3	2	5
Diffuse sclerosing GN	12	3	15	2	—	2
Systemic lupus erythematosus	51	5	56	—	1	1
Henoch-Schönlein nephritis	10	13	23	—	—	—
Miscellaneous	12	3	15	—	—	—
	560	172	732	66	21	87

Abbreviation is: GN, glomerulonephritis.

paraffin, serially sectioned and stained with hematoxylin and eosin, and periodic acid-Schiff (PAS). For electron microscopy, renal tissue was fixed in osmic acid and embedded in epoxy-resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to examination. Electron dense deposits were evaluated according to their location: mesangial, subendothelial, subepithelial, or intramembranous.

Immunofluorescence. Direct immunofluorescence method was used with fluoresceinated monospecific antisera to human IgG, IgM, IgA, C3 and fibrinogen purchased from Dako Corporation (Santa Barbara, California, USA). The amount of fluorescence was graded −, 1+, 2+, 3+, 4+.

Indirect immunofluorescence testing was carried out for HBsAg using the rabbit anti-HBs reagents as the primary antibody. The anti-HBs sera were made two times: the first batch was prepared from No. 1 rabbit immunized with purified HBsAg (Green Cross Corp., Yongin, Korea). The second one was separately made from No. 2 and 3 rabbits two and a half years later, using the HBsAg from the same company. The positivity for anti-HBs was checked by RIA and its titer was expressed in RIA units. The second reagent was FITC-labeled, swine anti-rabbit immunoglobulins (Dako Corp.). For negative control studies, frozen sections of kidney from seven cases of idiopathic MN and ten cases of diffuse proliferative lupus nephropathy were used. There was a positive reaction when the antisera from No. 1 rabbit were used as the first reagent, whereas no reactivity was observed when the primary antibody from No. 2 and 3 rabbits and FITC-labeled antibody were used in sequence or when the FITC-labeled antibodies were used alone. Therefore, for our study only antibodies from No. 2 and 3 rabbits were employed as the primary reagent. Frozen liver sections of three patients known to contain cytoplasmic HBsAg served as positive controls; many hepatocytes showed cytoplasmic staining, following the indirect immunofluorescence procedure with the rabbit anti-HBs preparation as the first reagent and FITC-labeled anti-rabbit IgG or FITC-labeled anti-rabbit IgG $F(ab')_2$ (Cappel Lab.) as the second reagent (Fig. 1).

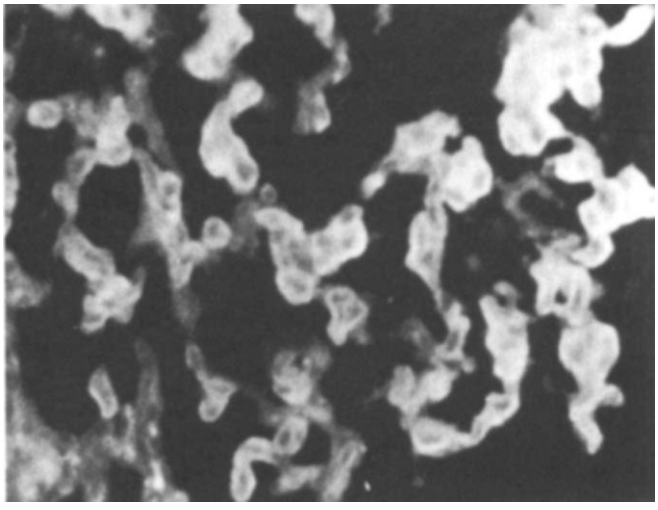

Fig. 1. *Numerous hepatocytes containing HBsAg in their cytoplasm detected by indirect immunofluorescence.* × 350

Results

Eighty-seven of the 732 patients with glomerular lesions were HBsAg carriers: 66 patients (11.8%) out of 560 adults and 21 (12.2%) out of 172 children had seropositivity (Table 1). Forty of the 87 HBsAg positive patients were excluded from the group of HBV-associated nephropathy: six had epithelial cell disease, six focal segmental glomerulosclerosis, sixteen IgA nephropathy (IgA N), four postinfectious glomerulonephritis (GN), five mild focal nonspecific GN, two diffuse sclerosing GN, and one showed membranous lupus nephropathy. Twenty-nine HBsAg carriers with MPGN and 18 seropositive patients with MN were diagnosed as having HBV-associated nephropathy, and these patients formed the basis of this study.

HBV-associated membranoproliferative glomerulonephritis

Clinical features at the time of biopsy. Of the 29 patients, only one was a child (Table 2). There was a preponderance of males in the 28 adults, who had a mean age of 30 years. In fifteen patients HBsAg carriage was detected at the time of renal biopsy when one to 15 years had elapsed since the onset of renal disease, while the development of renal abnormalities coincided with the discovery of HBs antigenemia in 12. Only two patients had known HBs antigenemia or chronic liver disease ten months to three years earlier than the onset of renal

Table 2. Clinical data in patients with hepatitis B virus-associated membranoproliferative glomerulonephritis (MPGN) and membranous nephropathy (MN) at time of biopsy

	MPGN		MN	
	Adults $N = 28$	Child $N = 1$	Adults $N = 10$	Children $N = 8$
Age *years*	30.3 ± 7.3	13	40.2 ± 10.5	7.4 ± 4.3
Range	16–45		23–50	2–14
Sex *M:F*	23:5	0:1	9:1	5:3
Mean duration of disease before biopsy *months*	31.1	5	14.7	2.8
Range	0.5–180		0.2–54	0.5–9
Serum				
HBsAg+	28/28 (100)	1	10/10 (100)	8/8 (100)
HBeAg+	16/19 (84)	1	6/9 (67)	6/6 (100)
Anti-HBs+	1/27 (4)	0	2/9 (22)	0/8
Anti-HBe+	1/14 (7)	ND	2/7 (29)	0/4
Anti-HBc+	23/24 (96)	1	8/8 (100)	6/6 (100)
Hypertension	13/28 (46)	0	1/10 (10)	0/8
Renal impairment	6/28 (21)	0	1/10 (10)	0/8
Proteinuria *g/24 hr*				
<1.0	5/28 (18)	0	0	3/8 (38)
1.0–3.4	8/28 (28)	1	3/10 (30)	3/8 (38)
≥3.5	15/28 (54)	0	7/10 (70)	2/8 (25)
Hematuria	21/28 (75)	1	4/10 (40)	8/8 (100)
Hypocomplementemia	11/26 (42)	1	2/10 (20)	4/7 (57)
Increased SGOT/SGPT	18/28 (64)	1	5/10 (50)	5/8 (63)

Figures in parentheses refer to percentage of positives. ND, not done.

Table 3. Ultrastructural features in patients with HBV-associated membranoproliferative glomerulonephritis (MPGN) and membranous nephropathy (MN)

	Number of positive cases		
	Subendothelial deposits	Subepithelial deposits	Mesangial deposits
HBV-associated MPGN ($N = 28$)	28	22	28
HBV-associated MN ($N = 17$)	4	17	12

Table 4. Results of immunofluorescence microscopy

	Number of positive cases					
	IgG	IgM	IgA	C3	Fibrinogen	HBsAg
HBV-associated MPGN ($N = 24$)	17	23	12	13	7	0 (11)
HBV-associated MN ($N = 16$)	16	13	2	14	5	0 (6)

Figures in parentheses refer to the number of patients in whom these tests were done.

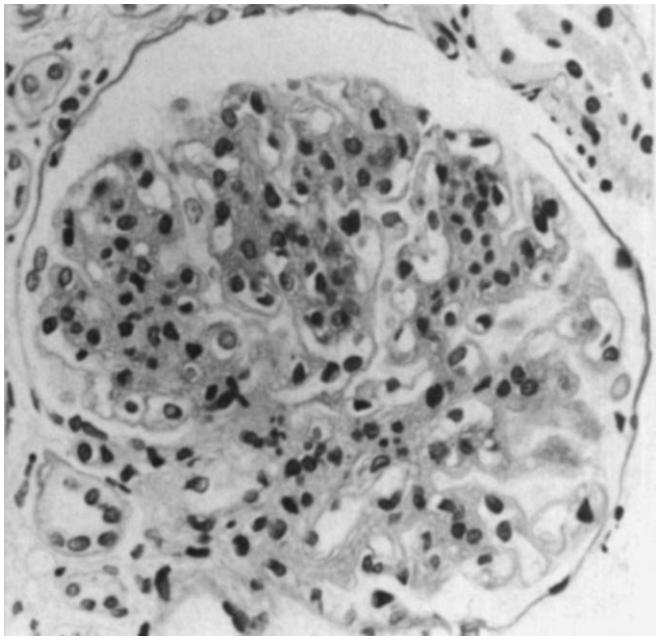

Fig. 2. *Hepatitis B virus-associated membranoproliferative GN showing prominent increase in mesangial substance and localized thickening of peripheral capillary loops.* PAS stained; × 430

disease. No patients showed signs of systemic lupus erythematosus (SLE). All the patients' sera were positive for HBsAg. Eighty-five percent of the 20 patients showed positive HBeAg, while the positivity for anti-HBs or anti-HBe was less than 8%, respectively. Hypertension, defined as blood pressure greater than 140/90 mm Hg, was present in 13 patients (44.8%). Renal insufficiency, diagnosed as serum creatinine in excess of 1.5 mg/dl, was noted in six patients (20.7%), mostly in mild degree; of the five patients who were followed from six months to two years two revealed the restoration of normal renal function at their last examination, whereas three exhibited persistently and slightly abnormal renal function. Proteinuria was observed in all, ranging from 0.6 to 19 g/day, and fifteen (51.7%) had nephrotic range proteinuria ≥ 3.5 g per day. One patient presented gross hematuria. Microscopic hematuria greater than 5 RBC per hpf was noted in 21 (72.4%). The serum complement C3 and/or C4 values were decreased in twelve patients (44.4%). Nineteen patients (65.5%) showed increased serum transaminase levels.

Pathological features. In all 29 patients, glomerular lobulation, mesangial sclerosis and hypercellularity, and thickening of peripheral capillary loops were present in great variability (Figs. 2 and 3). Segmental or global sclerosis was observed in 25 patients. Small crescents were rarely noted. One case showed segmental aneurysmal dilatation of capillary walls. Minimal to marked tubulointerstitial change was present in all. Ultrastructurally, there were massive circumferential subendothelial deposits and moderate to heavy mesangial deposits in all 28 patients studied, with subepithelial and/or intramembranous deposits in 22 (Table 3, Fig. 4). One patient had subepithelial deposits, resembling humps. Mesangial interposition affected stretches of the capillary walls in all biopsy specimens, and was occasionally circumferential. In some interposed mesangium, the deposits showed severe resolution. Microtubular myxovirus-like structures were present in glomerular endothelial cells in five patients. Of the 24 patients with immunofluorescent staining, 17 disclosed granular, usually confluent deposition of IgG in peripheral portions of glomerular capillary loops (Table 4). Twenty-three showed IgM deposition. IgA staining was noted in 12 and C3 in 13. Immunofluorescence for HBsAg was

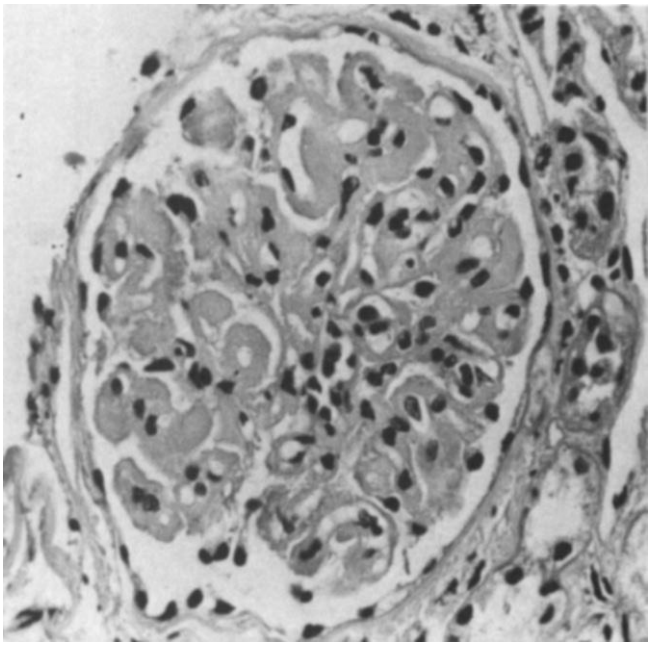

Fig. 3. *Hepatitis B virus-associated membranoproliferative GN showing considerably thickened capillary loops due to mesangial interposition and clumpy subendothelial deposits.* H&E stained; × 400

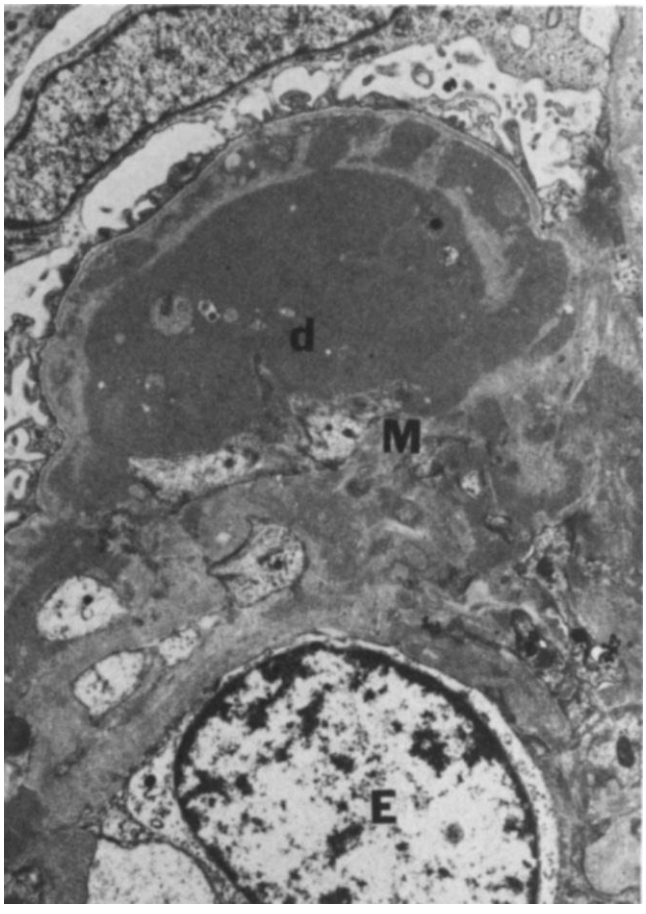

Fig. 4. *Electron micrograph from hepatitis B virus-associated membranoproliferative GN showing large subendothelial deposits (d) incorporated into mesangial matrix (M) as with moderate amounts of intramembranous and mesangial deposits.* E = endothelial cell nucleus. × 6,500

negative in all eleven cases studied. Liver biopsy was performed in five patients and showed macronodular cirrhosis in one patient and chronic aggressive hepatitis in four.

HBV-associated membranous nephropathy

Clinical features at the time of biopsy. Of the 18 patients with HBV-associated MN, eight were 14 years or younger. Clinical data at the time of the biopsy are summarized in Table 2, in which patients are grouped according to the age. Male predominance was only noted in adult group. Most patients were edematous at onset of the disease, whereas in two the presence of renal disease was found through the urinary examination in school children. Although not statistically significant, the mean duration of disease before biopsy was shorter in children. HBs antigenemia was frequently detected by systematic investigation for the diagnostic work-up of renal disease, whereas in two cases it preceded the onset of GN. None of the patients had clinical or laboratory evidence of SLE. All of the patients' sera were positive for HBsAg and anti-HBc. Eighty percent of the patients tested showed positive HBeAg. Hypertension was observed only in one patient and so was the case with borderline increase in serum creatinine concentration. Proteinuria was noted in all ranging from 0.4 to 10 g/day, the amount of which was significantly larger in adult group when compared to that in children ($P < 0.02$). No patients presented gross hematuria. Microscopic hematuria was found in all of the children and 40% of the adults; the frequency of hematuria in children was significantly higher than that in adults ($P < 0.02$). There were 35.3% of the patients who had low or borderline low C3 and/or C4 level. In ten patients serum transaminase levels were elevated.

Pathological features (Tables 3 and 4). In all 18 patients the renal biopsy by light microscopy showed diffuse thickening of the capillary walls. Nine cases exhibited mild to moderate mesangial cell proliferation with or without increase in matrix (Fig. 5). Ultrastructural studies were performed in 17 patients, showing diffuse glomerular subepithelial and/or intramembranous deposits in all patients and small amounts of mesangial deposits in twelve. In addition, a few isolated tiny subendothelial deposits were noted in four biopsy specimens (Fig. 6). The lesions were classified according to the criteria of Ehrenreich and Churg [30]: stage I was observed in four patients, II in eleven, III in one, and IV in one. The glomerular changes were not uniform in the same biopsy specimen, and were graded on the basis of the morphology of the majority of capillary loops observed. Microtubular virus-like structures in glomerular endothelial cells were found in four patients. Immunofluorescence studies revealed diffuse coarsely granular staining of IgG along the glomerular capillary wall of all 16 biopsy specimens, with lesser amounts of IgM in 13, IgA in two, and C3 in 14. HBsAg was negative in all six specimens tested. Liver biopsy was performed in one patient showing chronic aggressive hepatitis.

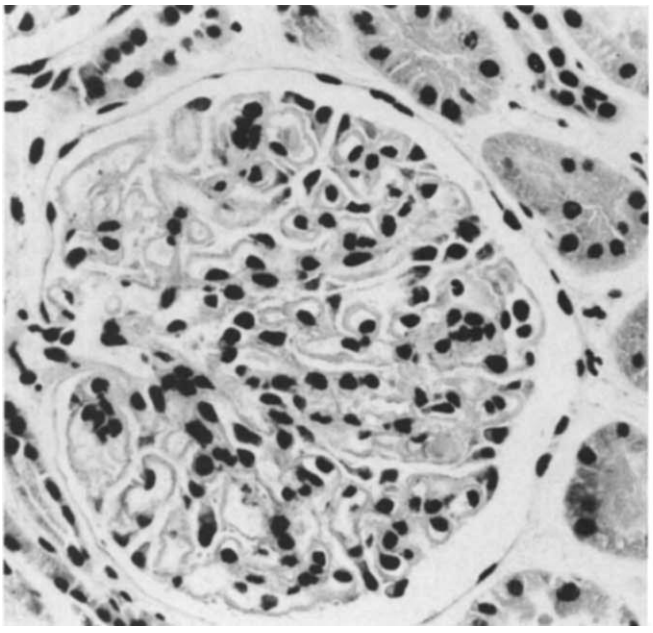

Fig. 5. *Hepatitis B virus-associated membranous nephropathy showing uniformly thickened capillary walls with mild increase in cellularity.* H&E stained; × 400

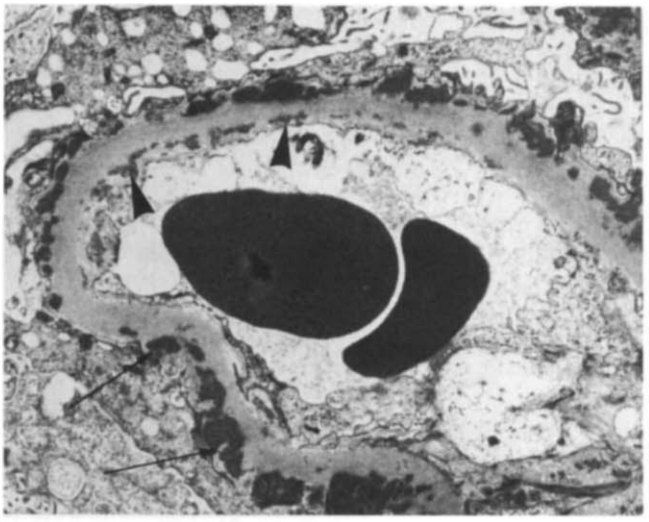

Fig. 6. *Electron micrograph from the same case as in Figure 5 showing several subendothelial deposits (arrow heads) in addition to numerous subepithelial deposits (arrows).* × 5,600

Discussion

In this study, we examined the frequency of HBsAg seropositivity in various forms of glomerular diseases and also investigated several clinical and pathological features of Korean patients who had HBV-associated nephropathy.

The frequency of HBs antigenemia in the patients with glomerular diseases, either primary or secondary, was 11.9%, showing no difference between adults and children. The HBsAg carrier rate of 11.9% is not different from 10 to 15% in the general population of South Korea including both adults and children. In contrast, several authors [8, 12, 24, 31–34] reported it was significantly greater among the patients with GN when compared to that in the general population or in hospital patients, suggesting that HBsAg carriers were predisposed to renal disease. Such a divergence of findings may be due to differences in case selection, methods of detection of markers of HBV, or geographic distribution.

Most of the previous reports [10, 14, 17, 19, 21, 33] emphasized the strikingly high incidence of MN associated with HBV occurring mainly in children. Although the number is small, eight (80%) out of ten children with MN in our series had HBsAg seropositivity partly supporting these notions. Nevertheless, the correlation between HBs antigenemia and MPGN in adults of our series was remarkable: 87.5% of the adults with MPGN were HBsAg carriers. This strong association of chronic HBs antigenemia and MPGN in adults has never been emphasized before and needs further confirmation, particularly in the regions having a high frequency of HBsAg carriers. The reasons for the different morphological predilection between adults and children are not evident, although some variations in immune responsiveness to the diverse HBV antigens could play a role.

Excluding the cases of HBV-associated MPGN in our series the frequency of idiopathic MPGN among primary glomerular diseases was less than 1.1%. The result is interesting in view of the recent reports that MPGN is disappearing in some European countries, which is speculated to be related to the decreased incidence of bacterial infections [35, 36]. The probable role of HBV for the pathogenesis of MPGN likewise suggests that through the control of this viral infection the frequency of MPGN may be markedly reduced, especially in the countries with a high prevalence of HBV carriers.

Several cases with IgA N related to HBsAg were reported [8, 11, 34]. However, the association between HBV infection and IgA N in this study is weak and may be incidental in view of the low frequency of HBsAg carriage in patients with IgA N, as were the cases with nonimmune-related glomerular diseases.

The morphological features showed no characteristic pattern in HBV-associated MPGN when compared to other patients with variable stages of MPGN type I without circulating HBsAg [37]. Those of HBV-associated MN were also indistinguishable from the cases with idiopathic MN, except for the frequent occurrence of small mesangial deposits with or without subendothelial deposits in the former, as has already been noted by some authors [23, 38].

There seems to be a striking male predominance in our adult patients with both forms of HBV-associated GN. However, we could not confirm the previous reports [10, 17, 19, 23, 33, 38] of a male preponderance in our pediatric cases.

Although the frequency of hypocomplementemia appears to be higher in adults with HBV-associated MPGN and children with HBV-associated MN when compared to that in adults with HBV-associated MN, the difference was not statistically significant. Previous reports of a relationship between hypocomplementemia and HBV-associated MN are not conclusive: Kleinknecht et al [19] believed the disturbances in serum complement levels were no more specific, whereas others [21, 23, 33, 38] regarded them as characteristic clinical features. In one-third of our patients with forms of MN, serum C3 or C4 values tended to be low. However, in the cases with HBV-

associated MPGN the frequency of hypocomplementemia was rather low when compared to that of idiopathic MPGN, suggesting hypocomplementemia in HBV-associated nephropathy is nonspecific.

Using indirect immunofluorescence technique we could not detect HBsAg in the glomeruli of our 17 patients with HBV-associated GN. As mentioned above, the results on HBsAg staining in glomeruli have been discordant. Various authors have all used their own antisera against HBsAg with direct or indirect immunofluorescence techniques. There appears to be a considerable difference in these antibodies as already shown by testing of our own reagents, and this may partially explain the marked differences in the results on HBsAg staining.

Even though HBsAg deposition was not demonstrable within the glomeruli, the etiologic role of HBV infection in MPGN or MN cannot be excluded. Several authors from Japan and the United States [18, 20, 22, 23, 27] have recently claimed that HBV-associated nephropathy may be mainly mediated by HBeAg. We also examined the presence of glomerular HBV antigens in three new cases with HBV-associated MPGN or MN using FITC-labeled $F(ab')_2$ fragments of monoclonal anti-HBs, anti-HBc and anti-HBe; glomerular HBeAg staining was noted in a patient with HBV-associated MN, while neither HBsAg nor HBcAg was seen in all of the three cases tested (unpublished data). Our demonstration of HBeAg supports the view of those Japanese authors [18, 20, 22, 23] that HBeAg is actually present in the glomerular deposits of patients with HBV-associated MN. Yet the possible responsibility of the different HBV antigens for the pathogenesis of GN needs further thorough investigation.

Acknowledgments

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