Yonsei Medical Journal
Vol. 23, No. 2, 1982

Incidence of Positive Serum Hepatitis B Surface Antigen and Its Antibody in Renal Diseases of Children

Pyung-Kil Kim, Kyo Sun Kim, Young Ho Kim, Jin Yong Lee and In Joon Choi*

Departments of Pediatrics and Pathology, Yonsei University College of Medicine*
Seoul, Korea

Serum HBsAg and anti-HBs were measured by radioimmunoassay in 384 patients with renal disease, admitted from January 1979 to December 1981 and in 167 controls. Among 384 cases of renal disease, 41 cases (10.7%) were positive for HBsAg, while only 8 out of 167 (4.8%) of the controls were positive, a difference which was barely significant ($P<0.05$). However in 159 biopsy-proven renal disease patients, 28 (17.6%) were positive for HBsAg, a difference which was highly significant when it was compared to the total control group ($P<0.005$). Seven of thirty-six (19.4%) cases with mesangioproliferative glomerulonephritis and 9 of 10 (90.0%) cases with membranous nephropathy were positive for HBsAg; when these were compared to the total control group the differences were highly significant ($P<0.01$ and $P<0.0001$ respectively). Serum HBsAg was also positive in the other renal diseases and anti-HBs was detected in some of the renal diseases but they were statistically not significant when compared with the non-renal controls. Glomerular deposits of hepatitis B surface antigen were identified in 5 of 8 biopsies in membranous nephropathy. The HBsAg and anti-HBs prevalence rate in the family members of the patients with renal diseases were 34.1% and 29.3% respectively.

Key Words: Hepatitis B Surface Antigen, Renal Disease

Korea is one of the endemic areas for viral hepatitis. The occurrence of hepatitis B surface antigen (HBsAg), a marker for hepatitis B virus (HBV), would be expected to be high in the Korean population as compared to Western countries where viral hepatitis is sporadic (Chung et al., 1971; Kim, 1975; Suh and Kim, 1978). Hepatitis B virus can cause not only hepatitis but other organ disease and an association between hepatic and renal disease has been established (Helwig and Schutz, 1932). The evidence indicates that the pathogenesis of most human glomerulonephritis involves the deposition of circulating antigen-antibody complexes in the glomeruli (Choi, 1978).

These findings suggest that persistent HBV antigenemia may activate antigen-antibody complex and complement systems, and induce

Received December 24, 1982

deposition of immune complex into various organ systems similar to the pathogenesis of chronic liver disease (Baldus and Summerskill, 1975).

In 1971, Combes *et al* investigated a patient with post-transfusion hepatitis B who developed membranous nephropathy. An immunofluorescent study of his kidney tissue revealed glomerular deposits of IgG, C_3 and HBsAg in a pattern characteristic of immune complex deposition, and they suggested that HBsAg may induce membranous nephropathy. Additional cases suggesting a strong relationship between HBsAg and glomerulonephritis were reported by Vos *et al* (1973), Brzosko *et al* (1974) and Nagy *et al* (1978).

The present authors therefore investigated further the frequency of HBsAg and anti-HBs in serum, and HBsAg in kidney biopsies from children with various forms of glomerulonephritis without clinically apparent liver disease in Korea, where viral hepatitis is endemic and the prevalence of HBsAg among apparently healthy people is high.

METHODS

From January 1979 to December 1981, 384 children under 15 years of age presenting with hematuria, proteinuria or both were examined. The 167 controls were children with disease other than liver disease and who had no history of blood transfusion.

To confirm the diagnosis, routine laboratory tests and renal function studies were done, and percutaneous renal biopsy was performed on patients whose diagnosis was uncertain from the results of clinical and laboratory examination.

Serum HBsAg and anti-HBs titer were measured by radioimmunoassay in all patients with renal disease (Overby *et al.*, 1973; Thorell and Larson, 1978).

RESULTS

Among 384 cases with renal diseases 41 (10.7%) were shown to be positive for HBsAg and 30 (7.8%) were positive for anti-HBs, whereas in 167 controls, 8 (4.8%) were positive for HBsAg and 14 cases (8.4%) for anti-HBs (Table 1).

Table 1. Incidence of positive serum HBsAg and anti-HBs in renal diseases and non-renal controls

	Total No.	No. of positive HBsAg[a]	%	No. of positive anti-HBs	%
Renal diseases	384	41	10.7	30	7.8
Non-renal controls	167	8	4.8	14	8.4

[a] P value<0.05

The positive rates of HBsAg and anti-HBs in various renal diseases are shown in Table 2. HBsAg was positive in 5.1% and anti-HBs was positive in 4.2% of 216 cases of acute poststreptococcal glomerulonephritis, but this was not significant when compared with the total control group ($P>0.05$). The positive rates of HBsAg and anti-HBs in 55 cases of minimal lesion nephrotic syndrome were 3.6% and 16.4% respectively ($P>0.05$). In mesangioproliferative glomerulonephritis, 7 (19.4%) were positive for HBsAg ($P<0.01$) and 5 (13.9%) were positive for anti-HBs ($P>0.05$). A very strong positive rate was observed in 9 out of 10 cases of membranous nephropathy ($P<0.0001$). In IgA nephropathy, 3 (37.5%) were positive for HBsAg ($P>0.05$) and 1 (12.5%) was positive for anti-HBs ($P>0.05$). Other forms of renal disease, such as Henoch-Schönlein purpura nephritis, congenital syphilis with nephrotic syndrome, hepatorenal syndrome, chronic glomerulosclerosis, chronic and acute renal failure, urinary tract

Table 2. Frequency of serum HBsAg and anti-HBs among 384 children with various renal diseases

Diagnosis	No. of patients studied	No. of positive HBsAg	%	P value	No. of positive anti-HBs	%	P value
Acute poststreptococcal glomerulonephritis	216	11	5.1	N.S.[a]	9	4.2	N.S.[a]
Minimal lesion nephrotic syndrome	55	2	3.6	N.S.	9	16.4	N.S.
Mesangioproliferative glomerulonephritis	36	7	19.4	<0.01	5	13.9	N.S.
Membranous nephropathy	10	9	90.0	<0.0001	0	0	
IgA nephropathy	8	3	37.5	N.S.	1	12.5	N.S.
Henoch-Schönlein purpura nephritis	11	1	9.1	N.S.	3	27.3	N.S.
Congenital syphilis with nephrotic syndrome	1	0	0		1	100(?)	N.S.
Hepatorenal syndrome	1	1	100(?)	N.S.	0	0	
Chronic glomerulosclerosis	2	1	50.0	N.S.	0	0	
Chronic renal failure	6	0	0		2	33.3	N.S.
Acute renal failure	11	3	27.3	N.S.	0	0	
Urinary tract infection	16	1	6.3	N.S.	0	0	
Hemorrhagic cystitis	11	2	18.2	N.S.	1	9.1	N.S.
Total	384	41	10.7	<0.05	30	7.8	N.S.

[a]N.S.: not significant

Table 3. Incidence of positive serum HBsAg and anti-HBs in biopsy-proven renal disease

Diagnosis	Cases of biopsy	HBsAg (+)	Anti-HBs (+)
Acute poststreptococcal glomerulonephritis	56	3	1
Minimal lesion nephrotic syndrome	35	2	6
Mesangioproliferative glomerulonephritis	37	7	5
Henoch–Schönlein purpura nephritis	11	3	3
Membranous nephropathy	10	9	0
IgA nephropathy	8	3	1
Chronic glomerulosclerosis	2	1	0
Total	159	28(17.6%)[a]	16(10.1%)

[a]P value<0.005 (when compared with control group)

infection and hemorrhagic cystitis were studied but the number of cases were too small to analyze statistically.

Among the 159 patients with biopsy-proven renal disease, 28 (17.6%) were HBsAg positive and 16 (10.1%) were anti-HBs positive(Table 3).

HBsAg was detected by immunofluorescent technic, in 5 biopsy specimens obtained from the 10 cases of membranous nephropathy (Table 4, Fig. 1).

Table 4. Tissue HBsAg in the glomeruli of the patients with the membranous nephropathy

Case No.	Age (years)	Sex	Serum HBsAg	Serum anti-HBs	Tissue HBsAg
1.	9	M			–
2.	12	F			N.D.[a]
3.	4	M			–
4.	2	M			+
5.	8	M			+
6.	8	M			
7.	6	M	+		+
8.	14	M	+		+
9.	9	M	+		+
10.	5	F	+		N.D.

[a]N.D.: not done

The positive rates of HBsAg among the family members of the patients with hepatitis B virus antigenemia were 18.2% in fathers, 37.5% in mothers and 42.9% in siblings. The total positive rate of anti-HBs was 34.1%. These are much higher than in the general population (Table 5).

Table 5. Positive serum HBsAg and anti-HBs in family members of patients

	Total No.	No. of positive HBsAg (%)	No. of positive anti-HBs (%)
Father	11	2 (18.2)	2 (18.2)
Mother	16	6 (37.5)	6 (37.5)
Siblings	14	6 (42.9)	4 (28.6)
Total	41	14 (34.1)	12 (29.3)

DISCUSSION

HBsAg is in the outer lipoprotein layer of hepatitis B virus. It appears 2-8 weeks before the clinical illness, and it persists for some weeks into convalescence. The presence of HBsAg in serum has been generally accepted as evidence

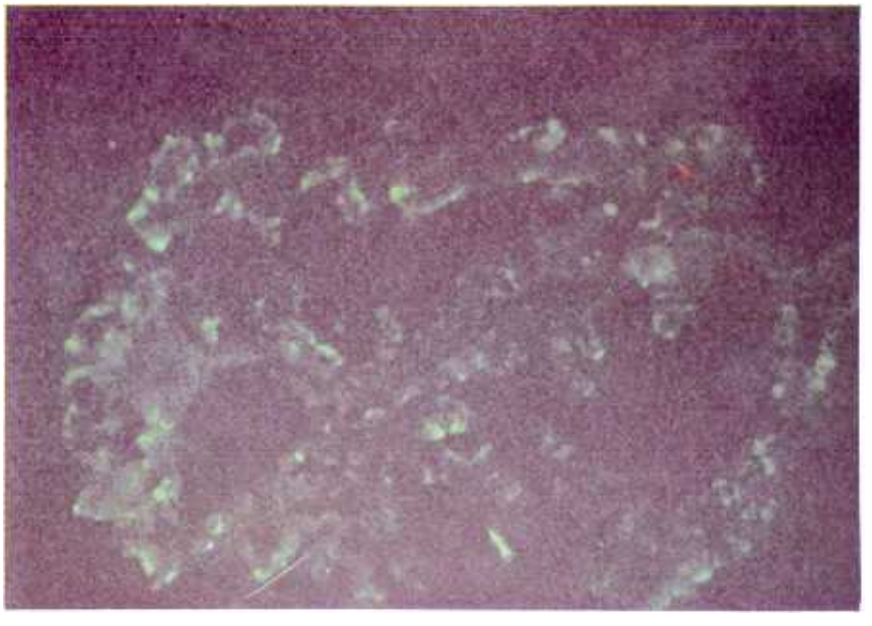

Fig. 1. Frozen section of kidney stained for HBsAg by the direct immunofluorescent technique (X 400). Numerous deposits of HBsAg are seen along the glomerular capillary walls in diffuse granular patterns.

of an on-going infection with hepatitis B virus or of a symptom-free carrier state (Blumberg *et al.*, 1970).

In Korea viral hepatitis is endemic and the prevalence of HBsAg is expected to be higher than in the U.S. or Europe (Chung *et al.*, 1971; Suh and Kim, 1978). The positive rate of HBsAg among commercial blood donors is 7-8% which is 3 times higher than in the U.S. where it is 2-3% (Kang *et al.*, 1977; Kim, 1975).

The HBsAg is now thought to be the cause of many immunologically-mediated diseases. The immune complex which contains hepatitis B antigen and antibody may induce tissue damage in many organ systems (Choi, 1978). Serum sickness-like syndrome is frequently observed in acute viral hepatitis, and HBsAg and anti-HBs containing cryoprotein were isolated from these patients. Furthermore, the immune complex was found as subendothelial deposits in renal biopsies of patients with acute viral hepatitis (Eknoyan *et al.*, 1972).

In 1974, Brzosko *et al* reported findings of immunoglobulins and complement in 32 of 52 unselected kidney biopsy specimens from children with clinical nephrosis and/or glomerulonephritis. Deposition with the composition and characteristics of viral hepatitis type B surface antigen/antibody complexes were identified in about 50% of these specimens. These findings strongly suggest that HBsAg immune complexes could play a primary role in a significant number of children with glomerulonephritis, who did not develop hepatitis clinically but who had been infected with viral hepatitis type B.

Vos *et al* (1973) investigated the South African Bantu and found a positive rate of HBsAg in renal pathology patients of 20.3% whereas in volunteer blood donors it was only 9.8%. This again suggests that the chronic carrier state of hepatitis B antigenemia may predispose to the development of renal disease.

The positive rate of HBsAg in adult renal disease in Korea has been reported as 56.3% (Kang *et al.*, 1977) and 25.3% (Kim, 1981). This is higher than in the normal control group (9.4%), and higher than the study by Nagy *et al* (1978) who found the positive rate of HBsAg was 13% in serum and 13.4% in kidney-biopsy specimens. In children, Brzosko *et al* (1974) detected hepatitis B surface antigen in the serum of 16 out of 32 (50%) cases of biopsy-proven glomerulonephritis. Takekoshi *et al* (1978) detected HBsAg in 3 of 170 and 4 of 100 children with glomerular and non-glomerular kidney disease respectively. Choi *et al* (1981) reported a 10.2% positive rate for HBsAg in 265 cases of renal disease. This last result is similar to the authors' study and supports the contention that there is a high positive rate in children compared to rates in control groups which have been reported to be 4.2% (Hong *et al.*, 1979), 4.0% (Chung), 5.0% (Choi *et al.*, 1981) and in the present study was 4.8%.

The presence of anti-HBs in serum has been generally accepted as evidence of past infection with hepatitis B virus or of passive or active immunity (Krugman et al., 1979). Knieser *et al* (1974) observed HBsAg in the kidney biopsy specimen but the serum was positive only for anti-HBs. Ozawa *et al* (1976) isolated the cryoprecipitable complexes of HBsAg and antibody from the serum of a boy who was anti-HBs positive and Brzosko *et al* detected HBsAg in 2 renal biopsies from patients who were anti-HBs positive. These findings suggested an on-going infection with hepatitis B virus in the presence of serum anti-HBs. The present authors' study revealed that the positive rate of anti-HBs was 30/384 (7.8%) but this was not a significant difference statistically when compared to the controls. If the positive rate of anti-HBs is added to the positive rate of HBsAg 10.7%, one fifth of the renal disorders were found to be

related to the hepatitis B virus.

The majority of the reports about glomerulonephritis associated with HBsAg concerned membranous nephropathy. In 1971 Combes *et al* firstly reported the association on HBsAg with membranous nephropathy in a 53-year-old man who had hepatitis after the transfusion of 4 pints of whole blood and 16 months later, developed the nephrotic syndrome. Immunofluorescent staining of kidney tissue revealed glomerular deposits of IgG, complement C_3 and HBsAg in a pattern characteristic of immune complex deposition. The patient was serum positive for HBsAg. Thereafter many studies about the association of HBsAg and membranous nephropathy were reported (Ainsworth *et al.*, 1974; Bajtai *et al.*, 1975; Bläker *et al.*, 1974; Brzosko *et al.*, 1974; Choi, 1978; Choi *et al.*, 1981; Gogan *et al.*, 1977; Kim, 1981; Kim *et al.*, 1977; Kleinknecht *et al.*, 1979; Knieser *et al.*, 1974; Kohler *et al.*, 1974; Nagy *et al.*, 1978; Ozawa *et al.*, 1976; Takekoshi *et al.*, 1978). In our study, membranous nephropathy was found in 10 cases and 9 of these were serum positive for HBsAg. The one negative HBsAg case was 12-year-old girl with membranous nephropathy who was admitted with the chief complaint of facial edema of 3 months duration. She had a negative history for systemic lupus erythematosus, syphilis, diabetes, exposure to toxic substances, neoplasm, renal vein thrombosis and systemic infection.

In most cases of the membranous nephropathy, HBsAg has been detected in subepithelial deposits by immunofluorescence (Ainsworth *et al.*, 1974; Bajtai *et al.*, 1975; Bläker *et al.*, 1974; Gogan *et al.*, 1977; Kim, 1981; Knieser *et al.*, 1974; Kohler *et al.*, 1974), and one case of acute immune-complex disease with glomerulonephritis in which glomerular fixed antibody was eluted, was shown to be HBsAg related (Ozawa *et al.*, 1976). We also found the deposition of HBsAg in the tissue of 5 out of 10 cases of membranous nephropathy. Takekoshi *et al* (1978) suggested that HBsAg associated glomerulonephritis may be due to a hepatitis B-associated antigen, but not necessarily to hepatitis B surface antigen.

Immune complexes of HBsAg weigh 3.5-4.7 X 10^6 dalton and most complexes do not cause glomerulonephritis inasmuch as they are cleared by mononuclear cells of the lung and blood. When immune complexes of HBsAg cause glomerulonephritis, it is due to a mesangioproliferation since it cannot cross the basement membrane (Kim, 1981; Stratta *et al.*, 1975). However, in three of eight patients with membranous nephropathy we were unable to detect HBsAg by tissue biopsy though the serum determination was positive.

Another disease reported to be associated with HBsAg is membranoproliferative glomerulonephritis (Brzosko *et al.*, 1974; Choi, 1978; Choi *et al.*, 1981; Kim, 1981; Kim *et al.*, 1977; Stratta *et al.*, 1975). This has been reported more often than membranous nephropathy in Korea (Choi, 1978; Kim *et al.*, 1977). The cases of mesangioproliferative glomerulonephritis (Brzosko *et al.*, 1974; Stratta *et al.*, 1975), acute glomerulonephritis, minimal lesion nephrotic syndrome (Kleinknecht *et al.*, 1979; Takekoshi *et al.*, 1978) and IgA nephropathy (Takekoshi *et al.*, 1978) have all been reported to be associated with positive serum HBsAg. In the present authors' study, HBsAg was positive in 7 of 36 cases (19.4%) of mesangioproliferative glomerulonephritis ($P<0.01$). Positive serum determinations were also found in acute poststreptococcal glomerulonephritis, minimal lesion nephrotic syndrome, IgA nephropathy, Henoch-Schönlein purpura nephritis, congenital syphilis with nephrotic syndrome, hepatorenal syndrome, chronic glomerulosclerosis, chronic and acute renal failure, urinary tract infection and hemorrhagic cystitis.

The present study was limited to an analysis of HBsAg and anti-HBs in hepatitis B virus-associated renal disease, but there have been reports associating HBcAg and HBeAg to renal disease as well. Takekoshi *et al* (1979) reported 2 cases of membranous nephropathy who carried both HBsAg and HBeAg in the serum. HBeAg was found to be deposited with IgG and C_3, but no deposition of hepatitis B surface antigen was detected. They suggested that HBeAg caused membranous nephropathy by inducing the formation and deposition of immune complexes. Slůsarozyk *et al* (1980) found HBsAg, HBeAg or both antigens to be positive in this condition.

The present authors detected more HBsAg and anti-HBs in the family members of HBsAg positive renal disease patients, (34.1% and 29.3% respectively) than in the general population. The rates were lower than those of Lee *et al* (1977) who found that the rate of HBsAg in siblings was 55.5% positive. Takekoshi *et al* (1978) reported 6 children with membranous nephropathy who had an HBsAg carrier mother and one had an anti-HBs positive mother. This high frequency of HBsAg or anti-HBs in mothers of patients with membranous nephropathy suggests that vertical transmission may contribute to the pathogenesis of membranous nephropathy.

The above findings suggest that further studies are required with regard to the association of renal diseases with HBsAg, anti-HBs, HBeAg and HBcAg. The association of hepatitis B virus infection with renal disease other than membranous nephropathy must be investigated in the future.

REFERENCES

Ainsworth SK, Brackett NC, Hennigar GR, Gevens LG: *Glomerulonephritis with deposition of Australia antigen-antibody complexes in the glomerular basement membrane. Lab Invest 30:369, 1974* (abstract)

Bajtai G, Ambrus M, Paal M, Nagy J, Deak GY: *Hepatitis B antigenemia associated with progressive cirrhosis and membranous glomerulonephritis. Lancet i:102-103, 1975*

Baldus WP, Summerskill WHJ: *Diseases of the liver (4th ed). Liver-kidney interrelationships. Philadelphia, JB Lippincott Co 1975, p. 445*

Blaker F, Hellwege HH, Kramer U, Thoenes W: *Membranous nephropathy and hepatitis B antigen. Lancet ii:955-956, 1974*

Blumberg BS, Sutnick AJ, London WT, Milman I: *Australia antigen and hepatitis. N Engl J Med 283:349-354, 1970*

Brzosko WJ, Krawczynski K, Nararewicz T, Morzycka M, Nowoslawski A: *Glomerulonephritis associated with hepatitis B surface antigen immune complexes in children. Lancet ii:447-481, 1974*

Choi IJ: *Hepatitis B antigens and extrahepatic diseases. Yonsei J Med Scie 11:38-47, 1978*

Choi Y, Lee WJ, Seo JK, Ko KW: *The incidence of HBs antigenemia in glomerular disease and control group in children. J Korean Ped Assoc 24:122-127, 1981*

Chung KS: *Hepatitis B virus infection after mass vaccination at school. Unpublished data.*

Chung WK, Kim BS, Choi JH, Ki CS: *Hepatitis associated antigen in various kinds of liver diseases in Korea. Korean J Gastroenterol 3:1-8, 1971*

Combes B, Stastny P, Shorey J, Eigenbrodt EH, Barrera A, Hull AR, Carter NW: *Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane. Lancet ii:234-237, 1971*

Eknoyan G, Gyorkey F, Dischoso C, Martinez-Maldonado M, Suki WN, Gyorkey P: *Renal morphological and immunological changes associated with acute viral hepatitis. Kidney Int 1:413-421, 1972*

Gogan MG, Graber ML, Connor DG: *Chronic active hepatitis and membranous glomerulonephritis. Am J Gastroenterol 68:386-391, 1977*

Helwig FC, Schutz CB: *A liver kidney syndrome. Clinical, pathological and experimental studies. Surg Gynec Obstet 55:570-580, 1932*

Hong SG, Soh JW, Oh JH: *Statistical and histological studies on HBsAg in elementary school children.*

J Korean Ped Assoc 22:433-442, 1979

Kang JK, Choi HJ, Yoo KJ: *Studies on HBsAg by radioimmunoassay in various diseases. Korean J Int Med 20:845, 1977 (abstract)*

Kim CY: *Occurrence of HBsAg in Korean populations and medical personnels. Korean J Int Med 18:705-710, 1975*

Kim SG: *The significance of serum HBsAg in various renal diseases. Korean J Int Med 24:199-214, 1981*

Kim YI, Lee JB, Lee WJ: *Glomerulopathies associated with chronic hepatitis - Histological reappraisal of glomerular lesions. Seoul J Med 18:106-116, 1977*

Kleinknecht C, Levy M, Peix A, Broyer M, Courtecuisse V: *Membranous glomerulonephritis and hepatitis B surface antigen in children. J Pediatr 95:946-952, 1979*

Knieser MK, Jenis EH, Lowenthal DT, Bancroft WH, Burns W, Shalhoub R: *Pathogenesis of renal disease associated with viral hepatitis. Arch Path 97: 193-200, 1974*

Kohler PF, Cronin RE, Hammond WS, Olin D Carr RI: *Chronic membranous glomerulonephritis caused by hepatitis B antigen-antibody immune complexes. Ann Int Med 81:448-451, 1974*

Krugman S, Overby LR, Mushahwar IK, Ling C, Frösner GG, Deihardt F: *Viral hepatitis, type B. Studies on natural history and prevention re-examined. N Engl J Med 300:101-106, 1979*

Lee MH, Chi YI, Kim CY, Hahn SS: *HBsAg prevalence rate in the family members of the chronic liver disease patients. Korean J Int Med 20:846-847, 1977 (abstract)*

Myers BD, Griffel B, Naveh D, Jankielowitz T, Klajman A: *Membranoproliferative glomerulonephritis associated with persistent viral hepatitis. Am J Clin Pathol 60:222-228, 1973.*

Nagy J, Bajtai G, Brasch H, Süle T, Ambrus M, Deak G, Hamori A: *HBsAg in renal disease. Lancet ii:315-316, 1978*

Overby LR, Miller JP, Smith ID, Decker RH, Ling CM: *Radioimmunoassay of hepatitis B virus associated (Australia) antigen employing ^{125}I antibody. Vox Sang Suppl 24:102-113, 1973*

Ozawa T, Levisohn P, Orsini E, McIntoshi RM: *Acute immune complex disease associated with hepatitis. Etiopathologic and immunopathologic studies of renal lesion. Arch Pathol Lab Med 100:484-486, 1976*

Slúsarozyk J, Michalak T, Nazarewicz-demezer T, Krawczynski K, Nowowlaeski A: *Membranous glomerulopathy associated with hepatitis B core antigen immune complexes in children. Am J Pathol 98: 29-39, 1980*

Stratta P, Camussi G, Ragni R, Vercellone A: *Hepatitis B antigenemia associated with active chronic hepatitis and mesangioproliferative glomerulonephritis. Lancet ii:179-199, 1975*

Suh DJ, Kim CY: *Detection of HBsAg and anti-HBs by immune adherence hemagglutination assay and passive hemagglutination assay in Korean patients with acute and chronic liver diseases. Korean J Int Med 37:796-819, 1978*

Takekoshi Y, Tanaka M, Miyakawa Y, Yoshizawa H, Takahashi K, Mayumi M: *Free "small" and IgG-associated "large" hepatitis B e antigen in the serum and glomerular capillary walls of two patients with membranous glomerulonephritis. N Engl J Med 300:814-819, 1979*

Takekoshi Y, Tanaka M, Shida N, Satake Y, Saheki Y, Matsumoto S: *Strong association between membranous nephropathy and hepatitis B antigenemia in Japanese children. Lancet ii:1065-1068, 1978*

Thorell JI, Larson SM: *Hepatitis B virus. Chapt 21 in: Radioimmunoassay and related techniques. Methodology and clinical applications. St Louis Mo 1978 CV Mosby Co. pp. 249-253*

Vos GH, Grobbelaar BG, Milner LV: *A possible relationship between persistent hepatitis B antigenemia and renal disease in Southern African Bantu. S Afr Med 47:911-912, 1973*