

Kidney International, Vol. 36 (1989), pp. 1103–1107

Membranous nephropathy in 52 hepatitis B surface antigen (HBsAg) carrier children in Taiwan

HEY-CHI HSU, CHIENG-YEN WU, CHING-YUANG LIN, GHI-JEN LIN, CHIUN-HUI CHEN,
and FU-YUAN HUANG

Departments of Pathology and Pediatrics, College of Medicine, National Taiwan University; Department of Medical Research and Pediatrics, Veterans General Hospital; and Department of Pediatrics, MacKay Memorial Hospital, Taipei, Taiwan, Republic of China

Membranous nephropathy in 52 hepatitis B surface antigen (HBsAg) carrier children in Taiwan. To elucidate the prognosis and the causative viral antigens of hepatitis B virus (HBV)-associated childhood membranous nephropathy (MN), the clinical course and glomerular HBV antigens were studied in 52 HBsAg carrier children with MN (40 boys, 12 girls). With Fab fragments of monoclonal antibodies, hepatitis B e antigen (HBeAg) was detected in the glomerular deposits in 41 (95%) of 43 cases but HBsAg and hepatitis B core antigen (HBcAg) in none. HBeAg was detected in sera from 43 (93%) of 46 children examined. These results suggest that HBeAg plays an important role in the development of MN in HBsAg carrier children. During the follow-up period (mean, 4 years), complete remission was found in 64% and 92% of the patients followed for one and seven years, respectively; only one child had mild renal function impairment. These findings suggest a favorable outcome of HBsAg-associated childhood MN. The patient's age, disease duration, amount of glomerular deposit, focal sclerosis and disease stage appeared to affect the clinical course. HBsAg seroconversion to HBsAg-negative occurred in seven cases, and all (100%) had quick remission in two years. In patients with persistent HBsAg carriage, serum HBeAg status alone did not correlate with remission rate and remission occurred usually before the HBeAg seroconversion to anti-HBe. These findings, together with the predominant horizontal infection in these children in contrast to the frequent vertical (perinatal) transmission from HBsAg carrier mothers in HBsAg carriers in Taiwan, suggest that factors other than HBeAg per se may also play important roles.

Since the association of hepatitis B surface antigen (HBsAg) with membranous nephropathy (MN) was demonstrated by Combes et al [1], hepatitis B virus (HBV) has been related to several types of glomerulonephritis [2–11], mostly MN [4–11]. This association is particularly striking in children and has been reported in countries with high or low prevalence of HBV infection. However, the nature of the antigen(s) involved and the clinical course of the HBV-associated childhood MN have not been clarified. All three major HBV-related antigens, that is, HBsAg, hepatitis B e antigen (HBeAg), and hepatitis B core antigen (HBcAg) have been implicated: HBcAg and/or HBsAg in Poland [5, 11], and HBeAg in Japan [4, 12, 13]. HBeAg exists in serum in two forms: free or complexed with immunoglobulin

[12]. Ito et al found that two patients who recovered from previous massive proteinuria were HBeAg-negative and anti-HBe-positive, and suggested that patients with HBeAg-mediated MN might remit as they seroconverted from HBeAg to anti-HBe [13]. However, the exact time relationship between HBeAg seroconversion and remission is not known.

Our study in Taiwan [7] failed to demonstrate the glomerular deposition of HBsAg in the majority of cases, and the role of HBcAg and HBeAg could not be determined because of the lack of appropriate antibody reagents [7]. Since then, case numbers have quadrupled and, with the availability of the Fab fragments of mouse monoclonal antibodies to HBsAg (anti-HBs), HBcAg (anti-HBc) and HBeAg (anti-HBe), we are now able to specifically determine the antigen(s) involved. Moreover, longer follow-up of the earlier cases also allowed a better understanding of the patients' outcome, in relation to the serum HBeAg/anti-HBe status.

Methods

Patients

In the renal biopsy series examined during January 1976 to March 1988 at the Department of Pathology, National Taiwan University Hospital, 463 were children with primary glomerular disease and under 17 years of age; 54 children (11.7%) had MN. Of these, 52 children (96.3%) with MN were positive for serum HBsAg and formed the basis of this study; 13 cases have been previously reported [7].

Morphologic and immunohistologic studies

Percutaneous kidney biopsies were divided into three parts for light microscopic, immunofluorescence and electron microscopic studies [7].

Detection of HBV antigens

Glomerular deposits were stained for HBsAg and HBcAg with polyclonal rabbit anti-HBs (Behringer, Marburg, FRG) and anti-HBc (Dakopatts, Santa Barbara, California, USA), respectively, followed by FITC-labeled goat anti-rabbit antisera. However, both gave false positive results in a few of MN without serum HBsAg, and the anti-HBc cross reacted with HBeAg in radioimmunoassay. Both were therefore used only for screening purposes.

Received for publication July 27, 1988
and in revised form May 13, 1989
Accepted for publication June 29, 1989

© 1989 by the International Society of Nephrology

Glomerular deposits of HBV-related antigens were tested further by direct immunofluorescence technique using FITC-labeled mouse monoclonal anti-HBs/a Fab' (IgG₃), anti-HBc/b F(ab')₂ (IgG_{2a}), and anti-HBe Fab (an 1:1 mixture of anti-HBe/a, IgG_{2a} and anti-HBe/b, IgG₁). These reagents were supplied by Y. Miyakawa and later obtained from the Institute of Immunology Co., Ltd, Tokyo, Japan; the individual determinants on HBsAg, HBcAg and HBeAg, to which the monoclonal antibodies are directed, have been characterized [14, 15].

For those cases without frozen tissue available for examination, the HBV antigens were examined in the deparaffinized sections by peroxidase-anti-peroxidase complex (PAP) technique [16] using the FITC-labeled monoclonal antibodies described above, followed by rabbit anti-mouse immunoglobulins (Cappel, West Chester, Pennsylvania, USA) or F(ab')₂ of rabbit antimouse IgG (Zymed Lab, San Francisco, California, USA).

Controls

To test the specificity of the FITC-labeled mouse monoclonal antibodies, six cryostat liver sections known to be positive for HBsAg were selected, including four positive also for nuclear and/or cytoplasmic HBcAg. The FITC-labeled anti-HBs stained HBsAg in all six specimens, and the anti-HBc stained HBcAg in the four cases positive for liver HBcAg. HBeAg was detected in none.

Negative controls included 24 cases of MN (22 adults and 2 children) and two children with mesangiocapillary glomerulonephritis (MCGN; including one with rheumatoid factor in serum), and all were negative for serum HBsAg. Five adults of MN who were positive for HBsAg but negative for HBeAg were also examined. Using Fab fragments of the monoclonal primary and secondary antibodies, glomerular HBeAg or other HBV antigens were detected in none of both groups by the direct immunofluorescent stain on frozen cryostat sections and/or by the indirect PAP stain on deparaffinized section. The positive control group included three adults with HBsAg and HBeAg in serum; all had glomerular deposition of HBeAg and one also had trace amount of HBsAg, but none had HBcAg.

Serologic tests

Serum HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were measured by radioimmunoassay (Abbott Laboratories, North Chicago, Illinois, USA).

Statistics

Fisher's exact test was used for statistical analysis.

Results

Clinical features

The ages of the 52 HBsAg-positive children with MN (40 boys and 12 girls) ranged from 2 to 16 years (mean \pm SD, 7.6 \pm 3.6 years), with a peak age of disease onset between two and five years (mean \pm SD, 5.9 \pm 3.1 years; Fig. 1). The disease duration before biopsy varied from 6 days to 10 years (mean \pm SD, 20.2 \pm 26.5 months). Nephrotic syndrome (NS) occurred during the course in 43 (83.3%), while seven had proteinuria alone and two, hematuria alone.

Serum HBeAg was detected in 43 (93%) of 46 patients. Serologic study was also performed in 47 patients followed.

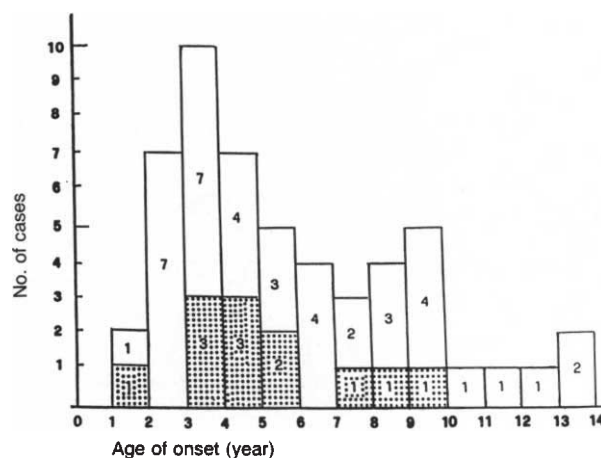


Fig. 1. Distribution of onset age in 52 children with HBV-related membranous nephropathy. Symbols are: (□) male, 40; (▨) female, 12.

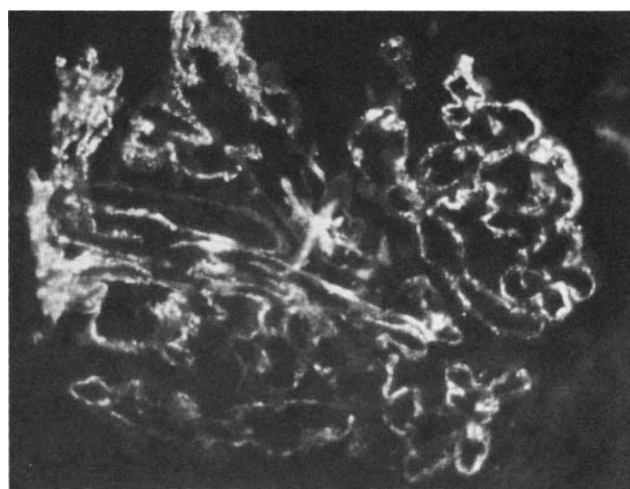


Fig. 2. Granular deposition of HBeAg along the glomerular capillary walls. Direct immunofluorescence stain for HBeAg with Fab fragments of mouse monoclonal anti-HBe, \times 720.

HBsAg was found in 3 (8.6%) of 35 mothers, and 14 (33%) of 43 siblings.

Pathologic features

The stages of glomerular changes according to the classification of Ehrenreich and Churg [17] were: I (4 cases), I-II (19 cases), II (16 cases), II-III (10 cases), III (3 cases), and IV (none). Focal glomerular sclerosis was found in 20%.

Detection of HBV antigens

HBeAg was detected in the glomerular deposits in all 26 cases examined by direct immunofluorescence (Fig. 2), but HBcAg and HBsAg in none. HBeAg, but not HBsAg or HBcAg, was also detected in deparaffinized section by indirect PAP technique in 15 of additional 17 cases (Fig. 3). HBeAg was detected in the second biopsies in two cases who remained positive for serum HBeAg, but not in one child who had cleared serum HBeAg.

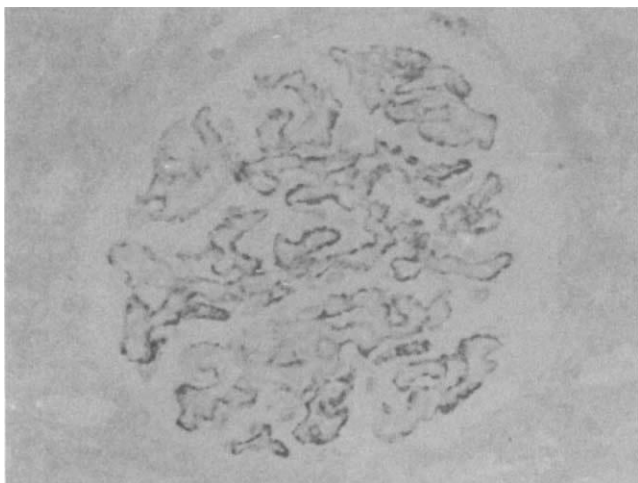


Fig. 3. Peroxidase-anti-peroxidase complex (PAP) stain for HBeAg. Fab fragments of mouse monoclonal anti-HBe and polyclonal rabbit anti-mouse antibodies were used to stain HBeAg in deparaffinized kidney section (2 μ thick) from a child with membranous nephropathy who had HBsAg and HBeAg in the serum. $\times 600$.

Table 1. Annual remission rates in relation to clinical and pathologic features in children with membranous nephropathy

	Complete remission %		
	2-yr	5-yr	7-yr
Total	30/47 (64)	34/41 (83)	35/38 (92)
Age at biopsy			
<6 yr	14/17 (82) ^b	14/16 (88)	14/14 (100)
>6 yr	16/30 (53) ^b	20/25 (80)	21/24 (91) ^d
Disease duration			
<12 months	21/28 (75) ^c	22/27 (82)	23/24 (96)
>12 months	9/19 (47) ^c	12/14 (86)	12/14 (86)
HBsAg and/or HBeAg seroconversion ^a			
HBsAg(-)	7/7 (100)	7/7 (100)	7/7 (100)
HBeAg(-)	9/14 (64)	10/13 (77)	10/12 (83)
HBeAg(+)	13/24 (54)	15/19 (79)	16/17 (94)
Histologic stage			
<I-II	16/20 (80)	18/19 (95)	18/18 (100)
II	10/15 (67) ^d	12/15 (80)	12/13 (92)
>II-III	4/12 (33) ^d	4/7 (57)	5/7 (71)
Subepithelial deposits			
1	9/10 (90) ^b	9/9 (100)	9/9 (100)
2-4	17/33 (52) ^b	21/28 (75)	22/25 (88)

^a HBsAg(-), HBsAg- and HBeAg-negative; HBeAg(-), HBsAg-positive and HBeAg-negative; HBeAg(+), HBsAg- and HBeAg-positive. *P* values are: ^b <0.05; ^c 0.0523; ^d <0.02.

Follow-up study

All the patients had been followed for 2 to 145 months (mean \pm SD, 47.56 \pm 35.45 months); 38 (73%) had received prednisolone therapy, but most did not respond. Adenine arabinoside (Ara-A) and thymic extract (thymostimulin, Serono Co., Italy) had been given to 10 cases. Four children did not receive any special therapy and all remitted in two years. The clinical course was analyzed among 49 children followed for a minimum of 12 months, the cumulative remission rate of the disease was high in seven years, and most patients remitted in two years (Table 1). At the last visit up to 12 years after renal biopsy, none had renal failure and only one had a mild elevation of serum creatinine (1.5 mg/dl). Two were recent cases and one lost to follow-up. The 14 patients who did not remit had mild proteinuria and/or microhematuria but none had NS.

Correlation of clinical parameters with disease course

The cumulative remission rate did not differ in relation to sex or age, but children of younger age or with a shorter disease duration remitted earlier (Table 1). During the course, seven children lost HBsAg and HBeAg, 12 became HBeAg-negative but HBsAg-positive, and 28 remained positive for both antigens. All seven children who became HBsAg-negative remitted in two years, significantly more frequent and earlier than those who remained positive for serum HBeAg (Table 1). Serum HBeAg alone did not correlate with remission and remission occurred in 12 of 13 children before they lost HBeAg; but 12 (75%) of 16 cases who did not remit remained positive for serum HBeAg.

Correlation of glomerular changes with outcome

The disease remitted earlier in patients with earlier disease stage, smaller amount of deposits or absence of focal sclerosis in glomeruli (Table 1).

Discussion

In this series, 52 (96.8%) of 54 children with MN were HBsAg carriers, confirming that HBV infection is one major cause of childhood MN in many countries [2, 4, 5, 7, 10, 11]. Although the association of HBV infection and childhood MN is well established, the causative antigen is not clear yet. HBsAg has been detected by some investigators using polyclonal anti-HBs [1-3, 5, 11, 18-21], but not by others [4, 6, 10, 22], and the reliability of these observations has been seriously challenged by Maggiore et al [23]. False positivity of HBV-related antigens in the glomerulus was also encountered with direct or indirect immunofluorescence stain using the whole immunoglobulins of the antibodies, as noticed by Maggiore et al [23]. This nonspecific staining was obviated by using the Fab fragments of all the antibodies. The current study could detect HBeAg in glomerular deposits in 41 (95%) of 43 patients examined, but HBeAg and HBsAg in none. It is also noticed that none of the patients experienced relapse of nephrotic syndrome after they lost serum HBeAg. These findings suggest that HBeAg plays an important role in the pathogenesis of MN, as noticed by other investigators [4, 12, 13]. This suggestion is supported by the appropriate molecular size of HBeAg. In free form, HBeAg measures 19,000 daltons; when complexed with immunoglobulin, it measures about 300,000 daltons [12]. In contrast, HBsAg is larger than 3 million daltons even without antibody, and HBeAg is not detected in serum. In experimental glomerulonephritis, immune complexes of 1 million daltons or less could precipitate in the glomerulus to induce typical MN [24]. Therefore, HBeAg appeared to be the most appropriate antigen for the development of HBV-associated MN.

Serum HBeAg positivity is high in children with MN in the present series (93%) and that (93%, or 14/15) from South Africa [10], but lower (19/31, or 61.3%) in Japan [13, 25, 26], and none in Zimbabwe [19]. The reasons for this wide range of HBeAg positivity are unknown, but may be related to the course of the

disease and the time when HBV markers were examined. Some children who had prolonged proteinuria, even after HBeAg seroconversion, were more often associated with more advanced and even permanent glomerular damage.

Children with idiopathic MN have a better prognosis than adults with MN [27, 28], but progression to renal failure is encountered in 10% of the children of Habib, Kleinknecht and Gubler [27] and in 27.3% in the series of Ramirez et al [29]. The current study with a mean follow-up period of four years demonstrated that HBV-related childhood MN had a cumulative remission rate of 92% in seven years, with only one patient showing mild renal function impairment. Except for one African [22] and one American child [8], renal failure has not been observed in children with HBV-related MN. The disease could remit spontaneously in the majority of cases, as reported by others [6, 10, 13, 22]. These findings suggest that HBV-associated MN in children runs a favorable course worldwide, even better than the idiopathic MN. However, prolonged course and permanent renal damage also exist. Little is known about the natural course of the disease. We found that several clinical, pathologic and virologic parameters were related to the disease course. Remission occurred more often and significantly earlier in children of younger age, with a shorter disease course, with smaller amount of glomerular deposits, and without focal glomerular sclerosis. The significantly longer course and lower remission rate in children with more advanced disease (stages II-III and III) and/or focal sclerosis suggest that permanent glomerular damage had already occurred in children with prolonged persistent proteinuria, as also noticed by Wyszynska et al [11]. HBeAg seroconversion to anti-HBe was frequently accompanied by remission, as noticed by the other investigators [13]. However, remission usually occurred before HBeAg seroconversion to anti-HBe, and even in the presence of serum HBeAg. Furthermore, we found that remission was related more closely to the HBsAg seroconversion; all the patients (100%) with HBsAg seroconversion to HBsAg-negative and anti-HBs-positive had remission in two years. These findings indicate that circulating HBeAg may be required for the maintenance of the immunopathologic process in the kidney [13], but its presence does not exclude the occurrence of a complete remission [10]. The reason for this discrepancy is not clear.

The present study reveals that HBsAg carrier children with MN are infected through the horizontal route; the predominant horizontal HBV infection in children with MN in a country where vertical transmission from HBsAg carrier mothers in the perinatal period is prevalent [30] is striking. Our results suggest that factors other than HBeAg per se, such as the patient's age, the route of infection, the capability of host immune response and the nature of the antibodies, particularly the anti-HBe, may also play important roles in the pathogenesis and course of HBV-related childhood MN.

Acknowledgments

We thank Professor Jacob Churg for reviewing this manuscript. Special thanks are offered to Professor Yuzo Miyakawa for providing some of the monoclonal antibodies used in this study, and for many helpful discussions. The work is partially supported by a grant from the National Science Council of the Republic of China (NSC77-0610-B002-71).

Reprint requests to Dr. Hey-Chi Hsu, Department of Pathology, National Taiwan University Hospital, Taipei, Taiwan, Republic of China.

References

1. COMBES B, STASTNY P, SHOREY J, ENGENBRODT EH, BARRERA A, HULL AR, CARTER NW: Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane. *Lancet* 2:234-236, 1971
2. BRZOSKO WJ, KRAWCZYNSKI K, NAZAREWICA T, MORZYCKA M, NOWOSLAWSKI A: Glomerulonephritis associated with hepatitis-B surface antigen immune complexes in children. *Lancet* 2:477-481, 1974
3. NAGY J, BAJTAI G, BRASCH H, SULE T, AMBRUS M, DEAK G, MAMORI A: The role of hepatitis B surface antigen in the pathogenesis of glomerulopathies. *Clin Nephrol* 12:109-116, 1979
4. TAKEKOSHI Y, TANAKA M, SHIDA N, SATAKE Y, SAHEKI Y, MATSUMOTO S: Strong association between membranous nephropathy and hepatitis-B surface antigenemia in Japanese children. *Lancet* 2:1065-1068, 1978
5. SLUSARCZK J, MICHALAK T, NAZAREWICZ T, DE MEZER T, KRAWCZYNSKI K, NOWOSLAWSKI A: Membranous glomerulopathy associated with hepatitis B core antigen immune complexes in children. *Am J Pathol* 98:29-44, 1980
6. KLEINKNECHT C, LEVY M, PEIX A, BROYER M, COURTECUISE V: Membranous glomerulonephritis and hepatitis B surface antigen in children. *J Pediatr* 95:946-952, 1979
7. HSU HC, LIN GJ, CHANG MH, CHEN CH: Association of hepatitis B surface (HBs) antigenemia and membranous nephropathy in children in Taiwan. *Clin Nephrol* 20:121-129, 1983
8. SOUTHWEST PEDIATRIC NEPHROLOGY STUDY GROUP: Hepatitis B surface antigenemia in North American children with membranous glomerulonephropathy. *J Pediatr* 106:571-578, 1985
9. DEL VECCIO-BANCO C, POLITO C, CAPORASO N, DEL GADO R, BUSACHI CA, COLTORTI M, DI TORO R: Membranous glomerulopathy and hepatitis B virus (HBV) infection in children. *Int J Pediatr Nephrol* 4:235-238, 1983
10. WIGGELINKHUIZEN J, SINCLAIR-SMITH C, STANNARD LM, SMUTS H: Hepatitis B virus-associated membranous glomerulonephritis. *Arch Dis Child* 58:488-496, 1983
11. WYSZYNSKA T, JUNG H, MADALINSKI K, MORZYCKA M: Hepatitis B mediated glomerulonephritis in children. *Int J Ped Nephrol* 5:147-150, 1984
12. 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
13. ITO H, HATTORI S, MATSUDA I, AMAMIYA S, HAJIKANO H, YOSHIZAWA H, MIYAKAWA Y, MAYUMI M: Hepatitis B e antigen mediated membranous glomerulonephritis. Correlation of ultrastructural changes with HBeAg in the serum and glomeruli. *Lab Invest* 44:214-220, 1981
14. USUDA S, TSUDA F, GOTANDA F, TACHIBANA K, NOMURA M, OKAMOTO H, IMAI M, NAKAMURA T, MIYAKAWA Y, MYUMI M: A solid-phase enzyme immunoassay for the common and subtypic determinants of hepatitis B surface antigen with monoclonal antibodies. *J Immunol Method* 87:203-210, 1986
15. TAKAHASHI K, MACHIDA A, FUNATSU G, NOMURA M, USUDA S, AOYAGI S, TACHIBANA K, MIYAMO H, IMAI M, NAKAMURA T, MIYAKAWA Y, MAYUMI M: Immunochemical structure of hepatitis B e antigen in the serum. *J Immunol* 130:2903-2907, 1983
16. HSU HC, LAI MY, SU JJ, CHEN DS, CHANG MH, YANG PM, CHEN CL, HSIEH HC: Correlation of hepaocyte hepatitis B surface antigen (HBsAg) expression with virus replication and liver pathology: Close association of marginal HBsAg with absence of both viremia and disease activity. *Hepatology* 8:749-754, 1988
17. EHRENREICH T, CHURG J: Pathology of membranous nephropathy. *Pathol Annu* 3:145-186, 1968
18. BLAKER F, HELWEGE HH, KRAMER U, THOENES W: Membranous nephropathy and hepatitis-B antigen. *Lancet* 2:955-956, 1974

19. KNIESER MR, JENIS EH, LOWENTHAL DT, BANCROFT WH, BURNS W, SHALHOUB R: Pathogenesis of renal disease associated with viral hepatitis. *Arch Pathol* 97:193-200, 1974
20. 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
21. SILVER MM, RANCE CP, MIDDLETON PJ, HUBER J: Hepatitis-B-associated membranous glomerulonephritis in a child. *Am J Clin Pathol* 72:1034-1039, 1979
22. SEGGIE J, NATHOO K, DAVIES PG: Association of hepatitis B (HBs) antigenemia and membranous glomerulonephritis in Zimbabwean children. *Nephron* 38:115-119, 1984
23. MAGGIORE Q, BARTOLOMEO F, L'ABBATE A, MISEFARI V: HBsAg glomerular deposits in glomerulonephritis: Fact or artifact? *Kidney Int* 19:579-586, 1981
24. GERMUTH FG, SENTERFIT LB, DREESMAN GR: Immune complex disease V. The nature of the circulating complexes associated with glomerular alterations in the chronic BSA-rabbit system. *John Hopkins Med J* 130:344-357, 1972
25. HIROSE H, KAZUMICHI U, KOJIMA M, TAKAHASHI Y, MIYAKAWA Y, MIYAMOTO K, YOSHIZAWA H, MAYUMI M: Deposition of hepatitis B e antigen in membranous glomerulonephritis: Identification by F(ab')₂ fragments of monoclonal antibody. *Kidney Int* 26:338-341, 1984
26. FURUSE A, HATTORI S, TERASHIMA T, KARASHIMA S, MATSUDA I: Circulating immune complex in glomerulonephropathy associated with hepatitis B virus infection. *Nephron* 31:212-218, 1982
27. HABIB R, KLEINKNECHT C, GUBLER MC: Extramembranous glomerulonephritis in children: Report of 50 cases. *J Pediatr* 82:754-766, 1973
28. CAMERON JS: Pathogenesis and treatment of membranous glomerulonephritis. *Kidney Int* 15:88-103, 1979
29. RAMIREZ F, BROUHARD BH, TRAVIS LB, ELLIS EN: Idiopathic membranous nephropathy in children. *J Pediatr* 101:667-681, 1982
30. STEVENS CE, BEASLEY RP, TSUI J, LEE WC: Vertical transmission of hepatitis B antigen in Taiwan. *N Engl J Med* 292:771-774, 1975