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Membranous Glomerulonephropathy Associated with Hepatitis B Virus Infection

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Abstract The association between hepatitis B virus (HBV) infection and the development of nephropathy remains controversial. The chronic carrier state of Hepatitis B Virus leads to the development of glomerulonephropathy, the commonest histological type being membranous nephropathy (MN), particularly in children. Other types of glomerulonephritis associated with HBV infection are less common. Spontaneous clearance of HBV antigens, particularly the HBeAg, leads to resolution of proteinuria. The isolation of immune complexes in the kidney suggests that the pathogenesis of the disease may have an immune-complex basis. Genetic studies suggest a possible genetic predisposition to the development of HBV-MN. Cellular immune mechanism studies suggest HBV-MN children seem to have an inadequate cellular immune response to HBcAg. Membranous nephropathy in patients with chronic HBV carriage is the result of an interaction of virus and host factors. Although the natural history of the disease suggests a tendency to remission with supportive therapy, there is considerable morbidity. Anti-viral therapy (interferon or lamivudine) accelerates clearance of the virus and proteinuria. Immunisation with HBV vaccination is the most effective tool to reduce the incidence of HBV-MN.

Key words Glomerulonephropathy; Hepatitis B Virus; Membranous

Introduction

In 1971, Combes et al were the first to describe a 53-year-old man with membranous nephropathy (MN) due to glomerular deposition of Australian-antigen-containing immune complexes.[1] The association of the chronic carrier state of hepatitis B virus (HBV) with membranous nephropathy is now well established, particularly in children, where the frequency of the HBsAg carrier state in MN correlates with the underlying prevalence of HBsAg in the general population. Other types of glomerulonephritis associated with HBV infection are less common. These include polyarteritis nodosa, membranoproliferative glomerulonephritis, mesangial proliferrative glomerulonephritis, minimal change disease, IgA nephropathy, focal segmental glomerulosclerosis, and crescentic glomerulonephritis. This article reviews the epidemiology, clinical presentation, pathology, pathogenesis and treatment of HBV-associated nephropathy.

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Epidemiology, Transmission and Presentation of Hepatitis B Virus Infection

The prevalence of hepatitis B virus (HBV)-membranous nephropathy (MN) varies geographically with the prevalence of the hepatitis B surface antigen (HBsAg) carrier state. The prevalence of HBsAg carriers is 0.3-1% in North America, 1% in western Europe, 7% in Africa, and up to 10% in South-East Asia where it is endemic.[2] One of the most important factors affecting prevalence is

the age of the patient when infected. Infection during infancy and early childhood carries the highest probability of development of a chronic carrier state. Acquisition of the HBV virus in children appears to be secondary to both vertical transmission from infected mothers and to horizontal transmission from infected siblings. Adults often have no known exposure, although individual cases have been reported in patients with a history of intravenous drug abuse, blood transfusion, homosexuality, and acquired immune deficiency syndrome.

The typical age of presentation with HBV-associated nephropathy is between 2 and 12 years, including nephritic/nephrotic syndrome or non-nephrotic proteinuria, and microscopic or rarely macroscopic hematuria. Hypertension is present in less than 25% of cases, and only rarely do children with HBV-MN manifest renal insufficiency. While idiopathic MN is associated with a slight male predominance, as many as 80-100% of children with HBV-MN are males.

Children with HBV-MN generally have no history or clinical evidence of active liver disease. Adults with HBV-MN are more likely than children to have a history of acute hepatitis, usually six months to several years prior to the onset of kidney disease. Despite a negative history of hepatitis, liver function tests and especially transaminases, are usually mildly abnormal, in contrast to idiopathic MN. The liver pathology in children with HBV-MN usually shows chronic persistent hepatitis or minimal abnormalities, but rarely affected children have been reported to suffer from chronic active hepatitis, cirrhosis, and even fulminating hepatitis. In contrast, the typical liver pathology in adults with HBV-MN is chronic active hepatitis, although cases have been reported in patients with other forms of acute and chronic HBV-induced liver disease. Immune complex deposits with early membranous and membranoproliferative changes have been found in autopsy cases with acute and subacute HBV hepatitis, chronic active hepatitis and cirrhosis despite the patients having had no clinical evidence of renal disease.

Diagnosis of HBV-associated Nephropathy

Presently, there are no internationally adopted criteria for diagnosis of HBV-associated MN. Criteria used within Mainland China are based on the diagnostic standards established in the Chinese Medical Association 1989 Symposium on HBV-associated MN, namely, (1) HBsAg and HBeAg are positive; (2) glomerulonephritis in the absence of systemic lupus erythematosus and other secondary glomerulonephritis and (3) HBV antigens were identified in the glomeruli, which is crucial for the diagnosis. The standards used overseas or in Hong Kong and Taiwan are similar to the aforementioned three criteria.[3]

Serology and Immunopathology of HBV-associated Nephropathy

The association between hepatitis B virus infection and the development of nephropathy remains controversial. More than 90% of patients will have evidence of acute hepatitis B infection with antigenaemia (positive HBsAg and HBeAg) and absence of antibodies, with the exception that anti-HB core antibody may be present. Approximately 60 to 80% of patients with HBV-MN will also have HBeAg and the remainder will have anti-HBe antibody in their sera.[4] Rarely patients may be positive for both HBeAg and anti-HBe antibody or for both HBsAg and anti-HBs antibody. Examples also exist where HBsAg has been found in the glomeruli of patients who lack HBsAg in their sera.[5,6] Some authors measured serum HBeAg circulating immune complexes (CICs) during the acute nephritic phase of HBV-MN and in the carrier stage of HBV. They found that the level of CIC was low in the HBV-MN patients, and absent in HBsAg+/HBeAg+ patients without HBV-MN, and in HBsAg+/HBeAg- asymptomatic carriers.[2,7]

In most studies, serum C3 and C4 levels have been reported as depressed in 15-64% of cases, although in some series complement levels were reported to be normal. Circulating immune complexes (CICs) have been reported in up to 80% of cases of childhood HBV-MN, whereas CIC are only rarely found in idiopathic MN. In one study, HBsAg immune complexes were found in 55% and HBeAg immune complexes in 44% of children with HBV-MN.

The light microscopic histological appearances are similar to idiopathic membranous glomerulonephritis, except that there may be more mesangial cell proliferation and areas of focal mesangiocapillary changes in some of the capillary loops. All cases stained positive for IgG, C3, IgM and complement. A subset of patients have mesangial IgA deposits. HBsAg and HBcAg were found inconsistently

in the glomeruli, whereas HBeAg was commonly found in subepithelial deposits. In HBV-MN, viral-like particles have been identified by electron microscopy (EM) in the subepithelial and subendothelial areas of the glomerular basement membrane (GBM) as well as within glomerular endothelial cells, epithelial cells, and mesangium.

Pathogenesis of HBV-associated Nephropathy

The pathogenic mechanism by which certain individuals with chronic HBV infection develop MN is unknown. Hepatitis B-associated membranous glomerulonephritis is probably immune complex mediated. HBsAg, HBcAg and HBeAg were all identified by immunofluorescent study (IF) in the glomerular capillary wall in HBV-MN. This would support a mechanism involving HBV antigen containing immune complexes (IC), presumably localising in the subepithelial space either as a result of passive trapping of CICs or due to local ICs formation at this site. Genetic factors and other factors may also play a role.

Circulating Immune Complexes (CICs) Containing HBV Antigen in the Glomeruli

Hepatitis B-associated membranous glomerulonephritis is probably immune complex mediated subepithelial space. HBeAg CICs have also been identified in 44% of children with HBV-MN, and appeared to correlate with disease activity. The potential importance of HBeAg in HBV-MN is further supported CICs containing HBV antigens have been demonstrated in many patients with HBV-MN.[8,9] HBsAg and HBcAg are large and anionic, and CICs containing HBsAg have been identified in HBV-MN with molecular weights of 2.5 to 3.6 x 10^6 daltons.[9] These are of a size that is unlikely to penetrate the basement membrane into the subepithelial space.[10] In contrast, HBeAg which is also anionic, induces anti-HBe antibodies that are largely cationic. HBeAg CICs are also small (2.5 x 10^5 daltons) and may thus be capable of localising in the subepithelial space by the observations that circulating HBeAg frequently correlated with the activity of the disease, and was the major antigen in the immune deposits. However, there is currently little experimental evidence to support a prefomed HBeAg CIC trapping mechanism that might account for the immune deposits seen frequently in HBV-MN.[10-12]

Local Immune Complexes (ICs) Containing HBV Antigen in the Glomeruli

Local formation of antigen-antibody complex has been well established to induce the diffuse subepithelial immune deposits and proteinuria characteristic of HBV-MN.[13] This process is usually initiated by a charge interaction between a cationic antigen or antibody and the highly anionic heparin sulfate proteoglycan present in the glomerular capillary wall. As discussed above, all three major HBV antigens (HBsAg, HBcAg, and HBeAg) are anionic, and only free HBeAg is of a small size that would be expected to penetrate the basement membrane. However, as anti-HBe antibodies are cationic (pI 5.8 to 10.2), sequential localisation of cationic antibody followed by anionic HBeAg could result in subepithelial ICs formation. This sequence has been shown experimentally to induce typical subepithelial immune complex deposits when cationic antibodies directed against various anionic antigens were studied.[14]

The Impact of HBV-DNA in the Pathogenesis of HBV-associated Nephropathy

A study by He et al[15] from China investigated the impact and significance of HBV-DNA in the pathogenesis of HBV-associated nephropathy. Renal tissue from 43 of 49 children with HBV-associated glomerulonephritis was examined for HBV-DNA by in situ hybridization assay. HBV-DNA was identified in 41 of the 43 cases (95.3%) and was distributed generally in the nucleus and cytoplasm of epithelial cells and mesangial cells of glomeruli, and epithelial cells of renal tubules. HBV-DNA was also present in renal interstitial tissue in some cases. The positive results from HBV-DNA in situ hybridization correlated well with HBV antigen assays. The duration of proteinuria in cases with HBV-DNA in renal tubules was much longer than in those with absence of HBV-DNA in renal tubules. The results suggested that the more extensive the existence of HBV-DNA in the nephron unit and interstitial tissue, the more severe the clinical manifestation. Zhou et al also reported that the existence of HBV-DNA usually coincides with the presence of HBV antigens.[16] This implied that HBV antigens, particularly HBcAg and its immune complex, could result from both antigens derived from local expression

and arriving from the circulation. The authors concluded that renal persistence of the HBV genome or genes could lead to the expression of viral antigens in this tissue and cause cytopathology and aberrant immune responses.

Genetic Factors

The increased frequency of MN in HBV infected patients may not be caused by either HBV or secondary liver disease, but rather may result from an underlying immunologic abnormality or genetic predisposition that increases the likelihood of these patients to develop both diseases independently of each other. Immunogenetic studies have demonstrated an increased prevalence of HLA B8 and DR3 in Europeans with idiopathic and drug-induced MGN, and an increased prevalence in DR2 in the Japanese patients. Patients with lupoid chronic active hepatitis also have an increased incidence of HLA B38 and DR3. A study reported the genetic study of 30 black children with biopsy-proven HBV-MN. HLA class I and class II antigen frequencies of the study subjects, when compared to controls that were healthy blood donors from the same population, showed a significantly increased frequency of HLA DQB1*0603 in patients with HBV-MN as compared to controls, suggesting a possible genetic predisposition to the development of HBV-MN.[17] In another study in Caucasian children with HBV-MN, there was also a significant increase in the frequency of HLA DQB1*0303 and DQB1*0603 alleles as compared to controls.[18] It was postulated that patients with HLA DQB1*0603 may be associated with poor clearance of HBsAg, resulting in deposition in the glomerular basement membrane and the initiation of immune-complex mediated glomerular damage.

Cellular Immune Mechanism

Defects of cell mediated immune response against hepatitis B virus have been reported.[7] Lin et al set out to elucidate why only some individuals with HBV infection developed HBV-MN. Using autologous HBcAg-expressing Epstein-Barr virus-immortalised lymphoblastoid cell lines as stimulator/target cells for the study of HBcAg-specific cytotoxic T lymphocytes, they found that HBV-MN patients had lower cytotoxic T-cell activity than did both HBV carriers and HBsAg-/HBsAb+ and HBeAg-/HBeAb+ children. From the in vitro cytokine production study of peripheral blood T cells after stimulation with HBcAg, they found that T-helper-cell-1-related interleukin (IL)-2 and IFN-gamma productions were very low in HBV-MN patients but T-helper-cell-2-related IL-10 production was higher in HBsAg+/HBeAg+ patients with HBV-MN than in those without HBV-MN. Based on these findings, they concluded that children with HBV-MN seemed to have an inadequate cellular immune response to HBcAg.[7]

Other Mechanisms

The prevalence of abnormal proteinuria in family members and household contacts of the index cases have been found to be higher than in community-based controls.[2,19] Children less than 5 years of age in the control group had a higher incidence of abnormal proteinuria compared with older children and adults. Some studies showed a lack of association between HBV carriage and proteinuria, which led some authors to the conclusion that HBV alone is not sufficient for the development of HBV-MN. It has been postulated that in HBV carriers, additional interaction between social-environmental conditions and possible factors in specifically vulnerable individuals, may be responsible for the development of HBV-MN.[2]

Prognosis

Most children clear their HBsAg from the blood with resolution of proteinuria without residual renal impairment. Uncontrolled studies suggested that children with HBV-MN have spontaneous recovery rates of 30-60% similar to those reported for children with the idiopathic form of MN. Progression to renal failure have been reported in patients who are not able to clear the HBV.[8] One study reported that two-thirds of the children with HBV-MN were in remission 3 years after diagnosis. This course is rare in adults and there is usually progression to renal failure over months to years.[2,20]

The natural history of most cases of HBV associated nephrotic syndrome in children without specific antiviral treatment is of gradual improvement. Remission of nephrotic syndrome parallels elimination of HBV antigens, especially HBeAg, with resolution of proteinuria occurring within six months of clearing the antigen (HBeAg) in the majority of cases.[20]

Treatment of HBV-MN

Corticosteroids and Immunosuppressive Therapy

The use of corticosteroid has not improved the course of renal disease and has actually contributed to the progression to the chronic active hepatitis. A prospective trial of corticosteroid in nephrotic patients with HBV-MN was conducted by Lai et al.[21] In this study, 8 patients with HBV-MN were treated with corticosteroids for 6 months and compared to similar historical control patients previously treated with diuretics alone. Corticosteroid therapy induced transient viral replication with increased serum concentration of HBeAg antigen and hepatitis B virus DNA. Histopathological examination of post-treatment renal biopsy in a single patient revealed histological progression, which did not support a protective value of corticosteroid therapy. Furthermore, the appearance of virus-like particles in the glomeruli after corticosteroid therapy supported the serological evidence of active viral replication.[22] Immunosuppression has not been an effective treatment, due to the importance of cell-mediated immunity in the elimination of the hepatitis B virus.

Anti-viral Therapies

There is always the possibility that patients may have spontaneously seroconversion without antiviral treatment. Spontaneous clearance of HBV antigen is slow. The reported rate of spontaneous remission (clearance of proteinuria with or without seroconversion) is 50% in 30 months.[20]

A priority of HBV research is the development of safe and effective anti-viral therapies.[23] Alpha-interferon (IFN-α) is a naturally occurring cytokine produced by B-lymphocytes, null lymphocytes and macrophages. IFN-alpha has anti-viral, anti-proliferative and immunomodulatory effects. In 2006, a meta-analysis[23] showed that most patients with HBV-related GN were successfully treated by anti-viral therapy, with the overall estimate for remission of nephrotic syndrome of more than 60%. In addition, the overall estimate for sustained remission of proteinuria induced by IFN therapy was 50%. IFN therapy has a beneficial effect in patients with chronic hepatitis B infection who respond by clearing HBeAg from serum. The reasons for the high rate of response in patients with GN remain unclear. Some patients with HBV-related GN have chronic renal insufficiency at diagnosis and IFN could help to restore the cell-mediated immunity depressed by uremia. Another possibility is that, patients with extra-hepatic manifestations of hepatitis B have an enhanced immunologic responsiveness. In 20 Chinese children (all HBeAg+/HBsAg+), treated with INF subcutaneously, three times per week for a year, all achieved sustained remission of proteinuria by three months. Sixteen seroconverted from HBeAg positive to anti-HBe positive, 12 of whom also cleared HBsAg.[11]

Lamivudine is a nucleoside analogue inhibitor of HBV-DNA polymerase, which has been proven to be safe and effective against hepatitis B in both adults and children, producing similar rates of HBeAg clearance to IFN therapy. Lamivudine has advantages over INF for HBV treatment, by having less frequent side effects (fever, headache, malaise, myalgia, as well as occasional blood dyscrasias, alopecia, and neuropsychiatric disturbance) and oral route of administration. In children, a dose of 3 mg/Kg/day produced drug concentrations and viral DNA inhibition similar to the standard 100 mg/day adult dose. A 4-week course of lamivudine is safe and effective in suppressing hepatitis B virus DNA in Chinese hepatitis B surface antigen carriers. No serious adverse events were observed. The suppression was >90% but reversible.[24] Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B antigen seroconversion rates. Up to two thirds of patients with moderately elevated pretreatment transaminases achieved HBeAg seroconversion after 3 years of therapy.[25] Prolonged lamivudine treatment can result in the emergence of drug resistance. Specific mutations (Val 552 Met and Met 528 Val) of the tyrosine, methionine, aspartate, aspartate (YMDD), nucleotide-binding locus of HBV polymerase result in a substantial decrease in sensitivity to lamivudine treatment.[26] In general, monotherapy with either lamivudine or interferon leads to sustained viral suppression in less than half the patients treated for chronic HBV infection. The recent trials of combination therapy using lamivudine with IFN have shown increased rates of seroconversion with minimal occurrence of drug resistant strains. In HBV-MN, antiviral treatment may be associated with resolution of proteinuria and HBeAg seroconversion, even when serum transaminases are normal. Patients seroconverted from HBeAg positive to anti-HBe antibody positive within three months of starting lamivudine (two years after presention) have shown a concurrent decline in the amount of proteinuria. Antiviral drugs may have additional therapeutic effects on glomerulonephropathy because of their immunomodulatory effects. The cell mediated immune response to HBV is defective in children with HBV-MN, and both IFN and lamivudine enhance this immune response.[27-29]

Prevention

Immunisation is important in HBV infection control programmes. Reports of the impact of mass HBV vaccination have documented a significant reduction in the prevalence of HBsAg carriage and an accompanying decline in the annual incidence of hepatocellular carcinoma in different populations. After the introduction of immunisation against HBV in July 1984 in Taiwan, an area of hyperendemic prevalence of HBV infection, HBV carriage in 6-year-old children declined from about 10% in the period 1981-1986 to between 0.9 and 0.8% in the period 1990-1994.[30,31] The incidence of hepatocellular carcinoma in children has also declined.[32] In a study conducted in Durban, South Africa, HBV-MN was used as an endpoint to assess the medium to long-term efficacy of HBV immunisation. The average annual rate ratio of HBV-MN showed a significant decline from 0.22 during the pre-immunisation period (1984-1995) to 0.03 during 2000-2001, 5 years after mass immunisation for HBV infection. The results of this study indicate that HBV vaccine is highly effective in reducing the incidence of HBV-MN.[33]

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