The frequency of hepatitis B surface antigen in membranous nephropathy in Black and White South Africans

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Summary
A series of 244 renal biopsy specimens obtained from patients with glomerulonephritis included 21 from patients with membranous nephropathy (MN). Of these patients 14 (10.7%) were Black, 1 was Indian and 6 were White (5.5%).

It is suggested that the high frequency of MN in Black patients may be associated with the high carrier rate (7-10%) of hepatitis B virus (HBV) in the Black population. Twenty of the 21 renal specimens were investigated for deposits of hepatitis B surface antigen (HBsAg) using the peroxidase-antiperoxidase method. HBsAg deposits were found in 13 of the specimens obtained from Black patients, in the specimen obtained from the Indian patient and in 1 of the 6 specimens obtained from White patients. HBV appears to be an important aetiological factor in MN in Black South African patients.

Membranous nephropathy (MN) is an important cause of the nephrotic syndrome in adults and a less common cause of this syndrome in children. The disease is characterized by the presence of immune complexes in the glomerular basement membrane. These are usually considered to be entrapped in the membrane, although recent work suggests that the complexes may be formed in situ. Although numerous antigens are associated with MN the antigenic components of the complexes are not identified in most cases. The postulated aetiological factors include neoplasms, autoimmune diseases, drugs and a spectrum of infective agents including viruses, bacteria and parasites. The cases of MN reported here are part of a larger retrospective study of 244 renal biopsy specimens obtained from patients with glomerulonephritis. The limited serological studies available suggested that hepatitis B surface antigen (HBsAg) might be common among the Black patients with MN in this series, and the peroxidase-antiperoxidase technique was therefore used to determine whether HBsAg was present in the glomerular basement membrane.

Material and methods
Twenty-one specimens from patients with MN were studied; 14 patients were Black, 6 were White, and 1 was an Indian. Specimens were obtained by open surgical biopsy (18 cases) or percutaneous needle biopsy (3 cases). Sections of paraffin-embedded tissue, 2-3 μm thick, were examined by light microscopy after staining with haematoxylin and eosin, periodic acid-methenamine silver and periodic acid-Schiff. In all, cases immunofluorescence studies for IgG, IgM, IgA, the C3 and C4 components of complement and fibrinogen were performed on cryostat sections after snap-freezing them in liquid nitrogen-isopentane slurry. Electron microscopic examination of glutaraldehyde-fixed, araldite-embedded tissue was performed using a Philips 300 electron microscope. Paraffin sections, pretreated with trypsin digestion, were examined by the peroxidase-antiperoxidase method, with rabbit antibody to HBsAg (Behring).

Results
The age, race and sex distribution of the 21 patients are shown in Fig. 1 and Table I. The male/female ratio was 2:1. MN was found to be more common among the Black patients with glomerulonephritis (14/131 (10.7%)) than among the Whites (6/109 (5.5%)).

Fig. 1. Age distribution of 21 patients with MN (14 Blacks, 6 Whites and 1 Indian).

<table>
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<th>TABLE I. SEX DISTRIBUTION OF PATIENTS WITH MN</th>
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Clinical features
All patients had marked proteinuria and 17 had the nephrotic syndrome. None had evidence of systemic lupus erythematosus or overt liver disease. Six patients had mild haematuria.

Documentation of possible aetiological agents was only available in a minority of cases. These included that of a 68-year-old White man with severe rheumatoid arthritis who had been treated with penicillamine and that of a 26-year-old White man...
with Hodgkin's lymphoma. Serological tests for HBsAg carried out on 4 of the Black males were found to be positive in 3 of them, but in 7 other patients (2 Black females, 3 White males, 1 Indian and 1 White female) tests for this antigen were negative. Two out of 10 patients examined for schistosomiasis had positive results. No evidence of streptococcal infection could be demonstrated in 9 cases, and 13 tests for syphilis proved negative.

Light microscopy
All cases fulfilled the pathological criteria of Ehrenreich and Churg. These include thickening of the glomerular basement membrane, absence of intracapillary or extracapillary cell proliferation, and demonstration of subepithelial and/or intramembranous deposits by light and electron microscopy. The features were those of stage II MN in 9 cases and of stage III MN in the remaining 12 (Fig. 2). The silver stain showed 'spikes' of basement membrane which separated the deposits (Fig. 3).

Immunofluorescence studies
The most striking feature was strongly positive fluorescence with IgG antiserum. In 20 of the 21 specimens granular deposits of IgG were seen in the peripheral capillary loops of the glomeruli (Fig. 4). A similar distribution of IgM was seen in 17 cases, and C3 deposits were seen in 15; IgA deposits were seen in only 3 cases, and mild deposits of fibrinogen were seen in 4.

Electron microscopy
In stage II MN subepithelial deposits extended into the basement membrane and were separated by projections of basement membrane (Fig. 5). In stage III MN the basement membrane was markedly thickened, and deposits were surrounded by new basement membrane. The deposits showed varying degrees of resolution.

Immunoperoxidase investigations for HBsAg
Tissue for this investigation was available in all but 1 case (a Black patient). The 13 specimens obtained from Black patients all had demonstrable HBsAg in the glomeruli. The specimen obtained from the Indian patient was also positive, but HBsAg was found in only 1 of the 6 specimens obtained from the White patients. The positive specimens showed a finely granular staining in the capillary loops, similar in distribution to the immunoglobulin deposits seen on immunofluorescence studies (Fig. 6). It also corresponded with the location of complexes seen on silver staining and electron microscopy. In all 3 cases in which HBsAg was found in the serum, HBsAg deposits were also demonstrated in the glomeruli.
Pathology of membranous nephropathy.

C. T. Biopsy Pathology with Membranous glomerulonephritis and hepatitis-B al.

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ment of immune complexes and immune complex-mediated disease associated with HBV. HBsAg may be concealed within cryoprecipitate or circulating immune complexes or may persist in glomerular complexes long after it has disappeared from the liver and the serum.

Fig. 6. Positive immunoperoxidase stain for HBsAg in basement membrane. The distribution of the complexes corresponds with that seen in Figs 3, 4 and 5 (x 250).

Discussion

In this series the Black and White patients differed markedly as regards both the frequency and the age distribution of MN. MN was more common in Black (10.7%) than in White patients (5.5%), and the Black patients with the disease tended to be younger than the Whites. The age distribution of the Whites is similar to that seen in European series, which show MN to be a disease mainly of the 5th - 7th decades, with fewer younger patients. Most of the Black patients in this series were aged between 15 and 34 years. The sex distribution shows a male predominance of 2:1, which corresponds to that found in other series.

It is suggested that the high frequency of MN in Black patients and the younger age at which it occurs in this group may be the result of an aetiological agent commonly found in young Black people. Hepatitis B virus (HBV) infection is common in the Black population of the RSA. The carrier rate is about 7 -10%, 11-12 which corresponds to that found in other series.

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In this series serological data were incomplete, but of the 13 patients investigated for HBsAg (6 of them being Black) only 3 Black patients had a positive result. None of the patients had clinical evidence of liver disease at the time of renal biopsy. They were probably chronic carriers, although the possibility of chronic active hepatitis cannot be excluded on the limited information available.

HBV infection results in a continued release of viral antigens into the circulation, providing an ideal setting for the development of immune complexes and immune complex-mediated MN. 19 HBsAg, 14 and in some cases hepatitis B core antigen 15 or hepatitis B e antigen 17 have been demonstrated in the immunofluorescence methods. The advantage of the immunoperoxidase technique is that it has made it possible to do retrospective studies on formalin-fixed, paraffin-embedded renal biopsy specimens.

The fact that glomerular HBsAg deposits were found in all 13 specimens obtained from Black patients in this series suggests that HBV infection is a most important cause of MN in Black South African patients. The high frequency of MN in Black patients is probably related to the high carrier rate of HBV in the Black population.

It is significant that HBsAg was demonstrated in the glomeruli in 4 cases in the absence of serological markers for HBV. HBsAg may be concealed within cryoprecipitate or circulating immune complexes or may persist in glomerular complexes long after it has disappeared from the liver and the serum.

REFERENCES