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# ROLE OF HEPATITIS $B_s$ ANTIGEN IN CHRONIC GLOMERULONEPHRITIDES IN NIGERIANS

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## Summary

Using enzyme-linked immunosorbent assay technique (Boehring Institute Laboratory), eighty-one adult patients were studied for hepatitis $B_s$ antigenaemia. Nine of the patients had asymptomatic persistent proteinuria, thirty-nine, nephrotic syndrome, and thirty-three had profuse proteinuria, azotoaemia and hypertension. The histopathology obtained in forty showed twenty-two with MCGN, four with focal glomerulosclerosis, three with proliferative glomerulonephritis, one with minimal change glomerulonephritis and ten with end-stage kidney disease. None of the patients had apparent clinical evidence of liver disease nor a past history of jaundice. One hundred and eighty apparently normal adults served as controls; 33.3% of the patients had positive hepatitis $B_s$ antigenaemia, in contrast to 6% ($P<0.001$) in the normal controls. Hepatitis $B_s$ antigenaemia was more prevalent in the groups with nephrotic syndrome and persistent asymptomatic proteinuria than in the group with advanced renal failure. Hepatitis $B_s$ antigenaemia was detected in all histopathologic forms but was most prevalent in the MCGN ($P<0.001$) which is also the more commonly encountered lesion. The implications of these findings are discussed.

## Résumé

Avec la méthode d'essai immunoabsorbant à enzyme (Boehring Institute Laboratory), quatre-vingt-et-un patients adultes sont examinés pour l'hépatite antigène $B_s$ dans le sang. Neuf des patients avaient la protéinurie asymptomatique persistante, trente-neuf le syndrome néphrotique et trente-trois avaient la protéinurie abondante, azotémie et hypertension. L'histopathologie de quarante patients a montré que vingt-deux avaient la MCGN, quatre avaient la glomérulosclérose focale, trois la glomérulonéphrite proliférative, un avec changement glomérulonéphrite minimale et dix avaient la maladie rénale terminale. Aucun patient n'avait une manifestation clinique apparente de maladie rénale ni antécédent de jaunisse. Cent-quatre-vingt adultes apparement normaux ont servi de contrôle. Un tiers des patients avaient l'hépatite antigène $B_s$ positive contre 6% ($P<0.001$) chez les controles normaux. L'hépatite antigène $B_s$ dans le sang a été plus remarquée chez les groupes atteints du syndrome néphrotique et la protéinurie asymptomatique persistente que dans le groupe qui accusait l'insuffisance rénale avancée. L'hépatite antigène $B_s$ a été détectée dans toutes ses formes histopathologiques mais elle était plus remarquée dans la MCGN ($P<0.001$) qui est la lésion plus fréquente. Les implications de ces constatations ont été étudiées.

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## Introduction

The predominant mechanism causing human glomerulonephritis (GMN) involves deposition

of circulating immune complexes in the glomerular capillary wall. In the majority of cases, the putative antigen(s) has not been characterised or is ill-defined thus rendering control and treatment of the disease elusive.

Certain agents have come to be regarded as playing a causal role. These include *Plasmodium malariae* (Gilles & Hendrickse, 1963), hepatitis B virus (Brzosko *et al.*, 1974), DNA (Koffler, Schur & Kunkel, 1967) and streptococcal antigen (Treser *et al.*, 1970). The ubiquitous nature of hepatitis B virus makes it a very important singular factor whose role can and should be evaluated across geographical and racial barriers. The aetiologic role of hepatitis B antigen ($HB_sAg$) in GMN has, however, not advanced beyond a speculative stage despite abundant data incriminating the agent. Brzosko *et al.* (1974) studied fifty-two children with GMN without apparent liver disease. Hepatitis B surface antigen ($HB_sAg$) was found in the glomeruli of eighteen of the patients. All eighteen had antibodies to the hepatitis B antigen in their serum while sixteen had $HB_s$ antigenaemia. Various glomerular histological features were described; namely membranous, mesangiocapillary (MCGN), endocapillary, and extracapillary GMN. Various other workers – including Combes *et al.* (1971), Kohler *et al.* (1974) and Hirschell *et al.* (1977) – have described one patient each with $HB_sAg$-associated glomerulonephritis.

The incidence of $HB_s$ antigenaemia in the adult African has been put between 6 and 10% (Williams *et al.*, 1972; Olusanya *et al.*, 1982; Zuzarte & Kasili, 1978). In such an endemic society this may also account significantly for the high incidence of chronic glomerulonephritis.

Amongst the South African Bantus with chronic GMN, Vos, Grobbelaar and Milner (1973) reported a high frequency (20.3%) of $HB_s$ antigenaemia, whilst in Nigeria, Obineche and Awunor-Renner, (1981) obtained a much higher incidence of 41% in their seventeen patients with acute glomerulonephritis.

Because of the reported association of hepatitis $B_s$ antigenaemia in chronic glomerulonephritis and its possible role as an aetiologic agent in the disease, we set out in this pilot study to investigate its frequency in sera from our patients with chronic glomerulonephritis, without apparent liver disease.

## Materials and methods

Eighty-one consecutive Nigerian patients aged 10–46 years (forty-three males, thirty-eight females), suffering from chronic glomerular disease, seen over a period of 2 years were studied. Nine of these had asymptomatic persistent moderately severe proteinuria of between 2–3 g/day; thirty-nine, nephrotic syndrome with serum creatinine being under 3 mg/100 ml; while the remaining thirty-three presented with gross proteinuria associated with severe azotaemia (serum creatinine over 3 mg/100 ml or blood urea over 100 mg/100 ml) and invariably a combination of one or more of the following features: significant hypertension (diastolic blood pressure, above 100 mmHg), oedema, and microscopic haematuria.

There was neither clinical evidence of liver disease, nor past history of jaundice. Only three of the patients had blood transfusion within 1 year of study. The patients had had symptoms of disease for a period varying from 6 months to 9 years.

Blood specimens were collected for routine laboratory investigations including serum creatinine, urea, albumin and globulin which were performed in all; serum transaminase levels (SGOT, SGPT) were obtained in thirty unselected cases. Five ml of serum from each patient and control was preserved at −20°C for periods ranging from 3 to 4 months before being tested for $HB_sAg$.

Renal histology was obtained in forty of the cases; in thirty of these, renal tissue was obtained by renal biopsy, whilst it was at autopsy in ten patients who had advanced renal failure.

Sera from 180 apparently normal adult Nigerians served as controls. Such controls included healthy undergraduate students and volunteer blood donors. They were aged between 15 and 40 years and included 100 males and eighty females. None had clinical evidence of liver disease nor a previous history of jaundice.

*Methodology*

The samples were examined for the presence of hepatitis B surface antigen ($HB_sAg$) using the enzyme-linked immunosorbent assay method with the commercially prepared kit (Boehring Institute Method). The assay was carried out in two stages as described below:

*Stage I.* The prepared tubes which had been coated commercially with the antibodies to $HB_sAg$ were allowed to warm up to room temperature. To each of the tubes was added 200 µl of each sample or each of the four known negative or each of two known positive controls provided in the kit. The tubes were then covered with an adhesive foil and incubated at 40°C for 90 min. The content of each tube was aspirated off and the tubes washed twice with Tween phosphate buffer solution to remove excess antigen. Two hundred µl of peroxidase-conjugated anti-$HB_sAg$ were added to each tube in turn and incubated at 40°C for 60 min. Each tube was completely aspirated four times with Tween phosphate buffer solution to remove excess anti-$HB_sAg$. Two hundred µl of phenylenediamine dihydrochloride dissolved in a mixture of hydrogen peroxide and citrate phosphate buffer solution was added to each tube and incubated for 3 min at room temperature avoiding exposure to strong light. One thousand µl of 0.5 M sulphuric acid was added to each tube to stop the reaction of the phenylenediamine dihydrochloride in peroxidase. The optical density (OD) was read within 60 min and any of the samples that had OD higher than that of the negative controls were presumed to be positive.

*Stage II.* Two hundred µl of each of the presumptive positive samples were added to two tubes in duplicate (1 and 2). To the first duplicate (1) was added 50 µl of commercially prepared anti-$HB_sAg$ serum while to the second duplicate (2), 50 µl of commercially prepared negative control anti-$HB_sAg$ serum was added. The tubes were incubated for 60 min at 40°C. The tubes were then reprocessed through the first stage of the method as described above.

A sample is specifically positive for $HB_sAg$ if the colour intensity in duplicate 1 is clearly lower than the colour intensity of duplicate 2 whereas it is negative if both tubes show the same colour intensity or if colour in both tubes is as weak as in the negative control tubes.

## Results

Eighty-one patients comprising forty-three males and thirty-eight females were studied along with 180 normal controls. Hepatitis $B_s$ antigenaemia was demonstrated in eleven (6%) of the controls whilst it was positive in twenty-seven (33.3%) of the patients, with a level of significance at $P<0.001$ (Table 1).

Table 2 shows the distribution of hepatitis $B_s$ antigenaemia across the age groups of the patients. Although the age group 20–29 showed an apparently higher prevalence than the rest, there was however no statistically significant difference in the prevalence between the age groups ($\chi^2 = 1.84$, 4 df, $P>0.5$).

Table 3 shows the distribution of hepatitis $B_s$ antigenaemia in relation to the clinical presentation. It was considerably high in both the asymptomatic persistent proteinuria and nephrotic syndrome with serum creatinine below 3 mg creatinine percent. There was however no statistically significant difference in its prevalence between the clinical groups of the disease ($\chi^2 = 3.72$, 2 df, $P>0.2$).

Table 4 showed that the patients with mesangiocapillary glomerulonephritis had a statistically significantly higher prevalence of hepatitis $B_s$ antigenaemia than the normal controls $P<0.001$ (Table 4). There was no significant difference in the prevalence of hepatitis $B_s$ antigenaemia in the patients with end-stage kidney, when compared with normal controls.

Hepatitis $B_s$ antigenaemia was not present in any of the three patients that had previous blood transfusion. A positive contact history was obtainable in ten of the eighty-one patients, only two of whom had positive hepatitis $B_s$ antigenaemia. None of the patients gave a history of previous jaundice.

Serum transaminases were only mildly raised in four of the thirty patients in whom this investigation could be performed. The prevalence of hepatitis $B_s$ antigenaemia was not significantly higher in patients with raised transaminases than in those without (Table 5, $\chi^2 = 1.15$, 1 df, $P>0.25$).

TABLE 1. Comparison of prevalance of hepatitis $B_s$ antigenaemia between patients and controls

| Population | *n* | Number with hepatitis $B_s$ antigenaemia | Percentage | Level of significance (*P*) |
|---|---|---|---|---|
| Normal controls | 180 | 11 | 6% | |
| Patients with chronic glomerulonephritides | 81 | 27 | 33.3 | <0.001 |

TABLE 2. Age–sex distribution and hepatitis $B_s$ antigenaemia

| Age | Male | Female | Number of patients | Number with hepatitis $B_s$ antigenaemia | Percentage |
|---|---|---|---|---|---|
| 10–19 | 11 | 12 | 23 | 7 | (30.4) |
| 20–29 | 21 | 8 | 29 | 12 | (41.4) |
| 30–39 | 6 | 13 | 19 | 5 | (26.3) |
| 40–49 | 3 | 5 | 8 | 2 | (25) |
| 50–59 | 2 | – | 2 | 1 | – |
| Total | 43 | 38 | 81 (100%) | 27 (33.3%) | |

$\chi^2 = 1.84$, 4 df, $P > 0.5$.

TABLE 3. The pattern of clinical diagnosis with respect to $HB_s$ antigenaemia

| Clinical diagnosis | Number of patients | Number with hepatitis $B_s$ antigenaemia | Percentage |
|---|---|---|---|
| Asymptomatic persistent proteinuria | 9 | 4 | 44.4 |
| Nephrotic syndrome with serum creatinine <3 mg% | 39 | 16 | 41.0 |
| Gross proteinuria with hypertension and serum creatinine ≥3 mg% | 33 | 7 | 21.2 |
| Total | 81 | 27 | 33.3 |

$\chi^2 = 3.72$, 2 df, $P > 0.2$.

TABLE 4. The distribution of histopathologic forms in forty patients and hepatitis $B_s$ antigenaemia

| Histopathology | Number of patients | Number with hepatitis $B_s$ antigenaemia | Comparison with prevalence in normal control level of significance (*P*) |
|---|---|---|---|
| Mesangiocapillary glomerulo-nephritis | 22 | 11 | <0.001 |
| Focal glomerulo-sclerosis | 4 | 1 | – |
| Proliferative glomerulo-nephritis | 3 | 1 | – |
| Minimal change glomerulonephritis | 1 | 1 | – |
| End-stage kidney, (Autopsy) | 10 | 1 | >0.5 |

TABLE 5. Serum transaminases and hepatitis B antigenaemia

| Serum transaminases | Number of patients | Number with positive hepatitis $B_s$ antigenaemia |
|---|---|---|
| Raised | 4 | 3 |
| Normal | 26 | 11 |

$\chi^2 = 1.15$; 1 df, $P>0.25$.



## Discussion

The high carrier rate of $HB_sAg$ in the general population in Africa has been well documented and thus our finding of 6% is in keeping with the observation of other workers (Williams *et al.*, 1972; Zuzarte & Kasili, 1978; Olusanya *et al.*, 1982). The hepatitis $B_s$ antigen has often been implicated in the pathogenesis of chronic glomerulonephritis. In the African series, Abdurrahaman *et al.* (1981) observed that eight of thirty children with the nephrotic syndrome had $HB_s$ antigenaemia, while Vos *et al.* (1973) obtained a frequency of 20.3% amongst South African Bantus with chronic GMN. There has also been increasing evidence arising from sporadic case reports (Combes *et al.*, 1971; Kohler *et al.*, 1974) that hepatitis $B_s$ antigen may be a significant pathogenetic factor in Caucasian series. However, from a larger study by Brzosko *et al.* (1974), it was observed that hepatitis B antigen immune complexes accounted for eighteen out of thirty-two cases of chronic GMN with immune complex in glomerular deposits, and sixteen of these had demonstrable circulating hepatitis B antigen and antibody.

Our finding of hepatitis $B_s$ antigenaemia in 33.3% of our cases of chronic GMN as compared with 6% in the control population ($P<0.001$) strongly suggests a significant association between it and the disease. The pattern of distribution of the hepatitis $B_s$ antigenaemia amongst the various clinical entities revealed that it is more prevalent amongst cases of nephrotic syndrome and asymptomatic persistent proteinuria than in

chronic GMN with azotaemia and hypertension.

The apparent relative difference in the prevalence rate of hepatitis $B_s$ antigenaemia amongst the various clinical varieties may be due to the different aetiopathologic entity each represents. The clinical entity described as asymptomatic persistent proteinuria runs a benign course, and is more often associated with focal and mild pathologic lesions in the glomeruli. On the other hand, the patients with advanced chronic GMN with azotaemia and hypertension represent an end-stage of a disease process. In the latter situation, caution is necessary when interpreting immunobiochemical data because of the interaction of many factors including uraemic toxins and cellular immunodepression.

The pattern of distribution of histopathological varieties amongst the forty cases in which the renal morphology was known, revealed that the majority (twenty-two of forty) had mesangiocapillary glomerulonephritis (MCGN), in keeping with the general experience in the tropics. Hepatitis $B_s$ antigenaemia was present in 50% of such cases, thus suggesting that it might play a significant role in this disease. It is however notable that hepatitis $B_s$ antigenaemia was also observed, though in varying proportions, amongst the histopathologic forms in this study as has been reported by several workers. Brzosko *et al.* (1974) reported eighteen cases of chronic GMN associated with $HB_s$ antigenaemia, in which the distribution of the histological pattern was as follows: endocapillary GMN (two cases), MCGN (twelve cases) membranous (two cases) and endo- and extracapillary glomerulonephritis (two cases) respectively. Both chronic membranous and mesangiocapillary histopathologic types have been frequently reported in hepatitis associated GMN (Kohler *et al.*, 1974; Hirschel *et al.*, 1977).

The greatest proportion of our cases were within second and fourth decades conforming with the observation of Oyediran and Akinkugbe (1970). There was a correspondingly high prevalence rate of $HB_s$ antigenaemia amongst this group thereby making them an important source of spread of the hepatitis-associated agent.

Parenteral infection with hepatitis B virus from blood transfusion was not a prominent feature; only three of the eighty-one had blood transfusion but the role of intramuscular injection which is sometimes indiscriminately administered would be difficult to evaluate until proper documentation of such.

It is tempting to conclude that in the absence of a previous history of jaundice and lack of any significant clinical or biochemical liver functional abnormality, a distinct pathogenetic pathway may account for the glomerulopathy induced by hepatitis B virus infection. It is indeed the consensus of opinion that while the liver damage induced by hepatitis B virus is due to cellular immune mechanism, extra hepatic manifestations like glomerular lesions, may be immune complex-mediated.

Our study has demonstrated an association between hepatitis $B_s$ antigenaemia and chronic GMN, bringing to mind a few speculations: (a) the possibility of a pathogenetic relationship between them; if so the hepatitis B virus may initiate the disease *per se*, or merely sustain the disease mechanism after it might have been initiated by other factors; (b) it may be an opportunistic infection in a host whose immune status has been depressed. In whatever capacity, $HB_s$ antigenaemia is certainly deleterious, and because it is increasingly becoming amenable to control measures its role needs to be clearly defined.



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