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HBV and proteinuria in relatives and contacts of children with hepatitis B virus-associated membranous nephropathy

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Background. Hepatitis B virus (HBV)-associated membranous nephropathy (HBVMN) is an important cause of childhood nephrotic syndrome in regions endemic for the virus, but little is understood of the biosocial context in which the disease develops. We evaluated HBV status and proteinuria in family members and household contacts of index children with HBVMN to test the hypothesis that HBV carriage and asymptomatic proteinuria are closely linked and may be causally associated.

Methods. Thirty-one black children with biopsy-proven HBVMN were the index cases. One hundred and fifty-two family members and 43 black household contacts were the subjects of the study. We assessed HBV carrier status by testing for HBV antigens and antibodies using enzyme-linked immunosorbent assays (ELISA) and for HBV DNA by using slot-blot hybridization and the polymerase chain reaction. Sequencing of the precore region of HBV was done in a subset of both index cases and subjects. Proteinuria was assessed by measuring the urinary protein/creatinine ratio.

Results. Seventy-two (37%) of the 195 family members and household contacts were HBV carriers, and 53 (27%) had a protein/creatinine ratio greater than the physiological limit. The frequency of abnormal proteinuria was not significantly different in those with [22 out of 72 (30.5%)] or without [33 out of 104 (32%)] HBV carriage. This lack of association remained when carriers were classified into those who were HBsAg positive only and those with active viral replication (HBsAg and/or HBeAg and/or HBV DNA; $P = 0.01$). Family members were more predisposed to HBV carriage than household contacts, but abnormal proteinuria was present with equal frequency ($P = 0.48$). Age had a significant impact on proteinuria, with children less than five years being more likely to have abnormal proteinuria ($P = 0.008$). The prevalence of abnormal proteinuria in family members and household contacts of the index cases was more than that in community-based controls. The 10 index HBVMN cases and the 14 family members and household contacts who were tested all had HBV of genotype A.

Conclusion. These results suggest that the family members and household contacts of children with HBVMN are at very high risk of HBV carriage; they also have asymptomatic proteinuria at a significantly higher rate than community-based controls. The HBV carrier status was not associated with proteinuria, a finding supported by peak prevalences of proteinuria in those under five years but no corresponding peak for HBV carriage. Proteinuria may indicate glomerular basement membrane dysfunction. Environmental and social factors may underpin development of these two covert disorders, but are insufficient to account for the index cases of HBVMN. The emergence of children with HBVMN from such households additionally depends on unidentified and possibly genetic factors.

Key words: childhood nephrotic syndrome, HBV carrier, asymptomatic proteinuria, viral infection, glomerular basement membrane.

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The available information indicates that genetic, social, economic, infectious, nutritional, and climatic factors make differing contributions to pediatric renal disorders [1]. Genetic causes may predominate in industrialized countries, whereas exposure to pathogens may be more important in the third world [1, 2]. In order to explore this subject further, we chose to study the settings in which the hepatitis B virus (HBV)-associated membranous nephropathy (HBVMN) emerges.

Epidemiological, clinical, and immunological evidence suggests a causal association between HBV carriage and the development of HBVMN [2, 3], although some investigators dispute this interpretation [3]. The pathogenetic mechanisms by which individuals with chronic HBV infection develop MN remain enigmatic, although a number of immunologic processes related to immune complex deposition have been implicated [4, 5]. Genetic factors may play a role [6]. Some evidence supports a genetic predilection for the persistence of HBV antigenemia and for protection against HBV infection [6]. The reasons for the diversity of clinical outcomes after HBV infection, some of which are subclinical, are not known.

To date, there is no substantial evidence showing strong associations between human leukocyte antigen (HLA) types and HBVMN.

Asymptomatic proteinuria, without other evidence of renal disease, is not infrequent in adults and children. The amount of protein excreted in the urine has been used as the most sensitive method to assess the significance and magnitude of renal damage [7]. Measurement of the protein/creatinine ratio on a single urine sample is accurate and provides physiologically relevant information, as it avoids collection errors [7], and above a certain threshold, the ratio is strongly indicative of glomerular dysfunction [2].

In this article, we report on the prevalence of asymptomatic proteinuria and the HBV carrier state among family members and household contacts of index children with HBVMN. The hypothesis we framed was that there would be a high level of clustering of HBV in the households of children with HBVMN, that HBV was causally related to the development of nephropathy, possibly on a genetic basis, and that those subjects infected with HBV would be more likely to have significant proteinuria resulting from more subtle damage to the glomerular basement membrane than that in the index cases.

METHODS

Thirty-one black patients, aged 2 to 13 years, with biopsy-proven HBVMN served as index cases. One hundred and ninety-five family members and household contacts of these index cases comprised the study group.

Index cases were recruited over a three-year period (1995 to 1997) from the Renal Clinic at the King Edward VIII Hospital (Durban, South Africa), which serves as the tertiary referral center for the region of KwaZulu/Natal, South Africa. Nephrotic syndrome (NS) in the index cases was diagnosed in accordance with criteria used by the International Study for Kidney Diseases in Children [8]. Clinical examination and appropriate investigations were done to exclude other secondary causes of NS.

The subjects in the study group (family members and household contacts) were all black. A careful note was made of the relationship of each member of the study group to the index case. Once the index cases were recruited into the study, family members and household contacts were evaluated once during the study period. History and clinical examination were used to detect concurrent illnesses in combination with appropriate laboratory testing. All subjects were assessed in particular for underlying renal disease by urinary dipstix analysis and culture, measurement of blood urea and serum creatinine levels, and estimation of their glomerular filtration rate using the creatinine transformation formula [9]. In addition, all subjects had liver function tests (including liver enzymes) done at the time of evaluation. All were tested for the hepatitis C virus, but none were screened for parasitic infections. Testing for human immunodeficiency virus was done only if there was clinical evidence of disease.

Urinary protein concentration in first morning urine samples was measured by the method of Pesce and Strande [10], and creatinine concentrations were measured with the Beckman creatinine analyzer [11] to determine the value of the protein/creatinine ratio in first morning urine samples.

Hepatitis B status was determined using third generation enzyme-linked immunosorbent assay (ELISA) (Auszyme® Monoclonal; Abbott Laboratories, North Chicago, IL, USA). In addition, all index cases had determination of HBV DNA by slot-blot hybridization (SBH) and nested polymerase chain reaction (PCR). One hundred and seventy-five (89.7%) family members and household contacts of the first 28 families were tested for HBV DNA by SBH or PCR (first-round and nested PCR).

Method of DNA extraction from serum samples

The QIAamp blood kit (Qiagen GmbH, Hilden, Germany), a method for nucleic acid purification and removal of amplification inhibitors, was used according to the manufacturer's instructions. A 200 μl aliquot of serum was incubated with Qiagen protease and buffer AL at 70°C for 10 minutes. Potentially infectious agents were inactivated by incubation at 95°C for 15 minutes following cell lysis. The lysate was applied to a QIAamp spin column, spun, washed three times with buffer AW, and finally eluted with 50 ml best-quality water (BQW). Known positive and negative sera and BQW were used as controls for the extraction procedure.

Method of DNA extraction from urine samples

Prior to DNA extraction, urine samples were concentrated 25 times using an Urifil-10 concentrator (Millipore, Bedford, MA, USA). DNA was extracted from 140 μl of concentrated urine using the QIAamp viral RNA purification protocol (Qiagen). One hundred and forty μl of urine were mixed with 560 μl buffer AVL containing carrier RNA and were incubated at room temperature for 10 minutes, and 560 μl of ethanol were added to the sample. The solution was applied to a QIAamp spin column and centrifuged at 8000 r.p.m. for one minute. The last step was repeated with the remaining solution. The QIAamp spin column was washed twice with buffer AW. The DNA was finally eluted in 50 μl BQW.

Polymerase chain reaction

Primers specific for the precore/core region were used for nested PCR (Table 1) [12]. The final PCR reaction

Table 1. Oligonucleotide primers used in the study

	Primer	Sequence	Position[a]	Size[b]
Outer primers	1730(+)	5′CTGGGAGGAGTTGGGGGAGG	1730–1747	314 bp
	2043(−)	5′CAATGCTCAGGAGACTCTAAGG	2043–2021	
Inner primers	1763(+)	5′GGTCTTTGTACTAGGAGGCTG	1763–1783	204 bp
	1966(−)	5′GTCAGAAGGCAAAAACGAGAG	1966–1946	

Symbols are: (+), sense; (−), antisense.
[a] Denotes nucleotide position of hepatitis B virus ayw (GenBank accession no. V00866) where the EcoRI cleavage site is position 1[16]
[b] Size of the polymerase chain reaction product in base pairs

mixture contained 0.02 Uml^{-1} Taq DNA polymerase (Promega, Madison, WI, USA), 200 μM each of the dNTPs, 1 μM of each primer, 50 mM potassium chloride, 10 mM Tris-HCl (pH 8.8), 1.5 mM magnesium chloride, and 0.1% triton-X 100. Two and one half microliters of target DNA were added to 22.5 ml of reaction mixture. The first-round PCR was performed in a programmable thermal cycler (Perkin Elmer, Norwalk, CT, USA) with the following three-step cycling profile: denaturation at 94°C for 30 seconds, annealing at 62°C for 50 seconds, and polymerization at 72°C for 50 seconds for a total of 40 cycles, followed by a final extension of 10 minutes at 72°C. For the second-round PCR, a 5 μl aliquot of the first-round amplification product was reamplified using inner primers in the cycling profile shown earlier in this article, except that the annealing temperature was decreased to 58°C.

All amplifications of serum and urine samples were performed with HBV-positive and HBV-negative controls. Sera positive for hepatitis B surface antigen (HB_sAg), hepatitis B e antigen (HB_eAg), and HBV DNA (by slot blot hybridization) were used as positive controls. To avoid cross-contamination and false-positive results, the precautions and procedures suggested by Kwok and Higuchi were strictly adhered to [13]. DNA extraction, PCR amplification, and electrophoresis were performed in physically separated venues. PCR controls to detect contamination consisted of BQW added to the PCR mixture instead of DNA and a water control interspersed between serum or urine samples.

Detection of amplified product

A 15 μl aliquot of the amplified product was electrophoresed on a 2% agarose gel. Bands of the appropriate size (314 base pairs with single PCR or 204 base pairs with nested PCR) were visualized under ultraviolet light after ethidium bromide staining. Each sample was tested in duplicate and read as positive or negative only if the two results agreed. Positive and negative controls were run in parallel with every assay. The specificity of the amplified band was confirmed by Southern blot hybridization using ^{32}P-labeled HBV DNA probe (HBV DNA in pBV325 vector, adr subtype, purified and supplied by Professor C. Bréchot, INSERM U 370, Institut Pasteur, Paris, France).

Slot-blot hybridization assay for HBV DNA detection

Hepatitis B virus DNA in serum or urine was detected using a SBH assay using the method of Zaaijer et al [14]. Briefly, 50 μl of serum were denatured in an equal volume of denaturing solution [200 mM ethylenediaminetetraacetic acid (EDTA), 10% sodium dodecyl sulfate, 20 mg ml^{-1} proteinase K, and 12.5 mM Tris-HCl] at 56°C for two hours. The samples were heated to 95°C for 10 minutes and chilled on ice. Two hundred microliters 20 × SSC were added to the denatured samples and centrifuged at 13,000 r.p.m. for 15 minutes. The clarified samples were blotted onto Hybond-N nylon membrane (Amersham Life Science, Buckinghamshire, UK) using the Bio-Dot® SF microfiltration apparatus (BioLab Laboratories, South Richmond, CA, USA). The blots were treated with 0.4 mol^{-1} NAOH, dried in an 80°C oven for two hours and hybridized using ^{32}P-labeled HBV DNA probe (Megaprime DNA labeling system; Amersham). Blots were hybridized using QuikHyb® Hybridization solution (Stratagene, La Jolla, CA, USA). Autoradiography was carried out at −70°C with intensifying screens and Kodak film (X-Omat; Sigma, St. Louis, MO, USA) for four days.

Determination of genotype

To determine the genotype, the precore region of HBV DNA extracted from serum samples was sequenced directly from PCR products. The PCR amplification mixture (5 to 10 μl) was pretreated with two hydrolytic enzymes: exonuclease I (10.0 U/ml), to remove sticky ends and primers, and shrimp alkaline phosphatase (2.0 U/ml), to remove nucleotides (Sequenase PCR product sequencing kit; United States Biochemicals, Cleveland, OH, USA). Enzymatic pretreatment was carried out at 37°C for 15 minutes, and enzymes were inactivated by heating to 80°C for an additional 15 minutes in a thermal cycler.

Sequencing was carried out using the Sequenase PCR kit, but with modifications of the methods by Casanova et al [15] and Galibert et al [16]. The annealing mixture contained 0.5 pmol DNA (10 to 90% of the amplified sample, depending on the PCR yield), 10 pmol primer (1763 and 1966R; Table 1), and BQW in a final 10 μl volume. The sample was denatured at 99°C and then

immediately placed in an ice/water bath for five minutes. The labeling reaction was carried out using 10^{-1} dilution of labeling mix and 5 μCi − ^{35}S-dATP for two minutes. Termination was carried out at 40°C for five minutes. The sequences were analyzed on an 8% polyacrylamide gel in glycerol tolerant buffer and autoradiographed using Kodak film (X-omat; Sigma). Mutations were recorded only when they were detected in both the forward and reverse sequences.

Community-based controls

One hundred and twenty-three control subjects, none of whom were family members or household contacts of children with HBVMN, had their protein/creatinine ratios determined. All controls were negative for HBV and retrovirus. All were tested for hepatitis C virus and found to be negative for anti-HCV. None had evidence of any coexisting illnesses. None were screened for parasites.

Ethical considerations and statistical analysis

Ethical approval was obtained from the Ethics and Professional Standards subcommittee (faculty of Medicine). Informed written consent was obtained from parents before entry into the study. Statistical analysis was done in consultation with the Institute of Biostatistics of the Medical Research Council.

Definitions

Nephrotic syndrome. Nephrotic syndrome (NS) was defined as edema, plasma albumin less than 25 g/liter, proteinuria of more than 40 mg/m^2/hr, or a protein (mg/dl)/creatinine (mg/dl) ratio of more than 2.0 [8].

Histologic features on renal biopsy to diagnose membranous nephropathy. Light and electron microscopy and immunofluorescence results were defined according to the criteria used by Coovadia, Adhikari, and Morel-Maroger [17].

Quantitation of urinary protein excretion by measurement of urinary protein (mg/dl)/urinary creatinine (mg/dl). The normal level was defined as less than 0.2 in children two years of age or older, and less than 0.5 in children less than two years of age. Abnormal proteinuria was 0.2 or more in children two years of age or older, and 0.5 or more in children less than years old (mild + moderate + severe). Mild proteinuria was 0.2 or more and less than 0.5 in children two years old or older. Moderate proteinuria was 0.5 or more and less than 2.0 in children two years of age or older. Severe (nephrotic range) was 2.0 or more in children two years old or older [18, 19].

Household contact. A household contact was defined as a person whose residence, at least for the last two years, was the same as the index child. Children younger than two years old were included if they had been living with the index child since birth and were genetically related [5].

Family members. Family members included parents, full siblings, half siblings, aunts, uncles, and grandparents.

Index case, family members, and household contacts (subjects). These were categorized by HBV status using ELISA, SBH, and PCR as follows:

(1) Category A: HBsAg positive only = HBV carriers;
(2) Category B: HB_sAg and/or HB_eAg and/or SBH and/or PCR positive = HBV carriers: active replication of HBV;
(3) Category C: Anti-HBc IgG positive only or all markers negative by ELISA but SBH or PCR positive for HBV DNA = carrier;
(4) Category D: Anti-HBc IgG positive with SBH and PCR negative = exposed;
(5) Category E: Negative by all markers of HBV = unexposed;
(6) Category F: ELISA negative, SBH and PCR not done = indeterminate.

Chronic liver disease. This includes patients with clinical evidence of liver disease (firm hepatomegaly ± signs of portal hypertension, growth retardation, and metabolic bone disease) who had normal or raised liver enzymes [20].

Hypertension in children. This is defined as blood pressure higher than the 90th percentile for age, as defined by the Second Task Force on Blood Pressure Control in Children on three separate occasions [21].

Hypertension in adults. This is defined according to the Sixth Report of the Joint National Committee on Presentation, Detection, Evaluation and Treatment of High Blood Pressure [22].

RESULTS

Index cases

Seventeen (55%) of the 31 index cases were of rural origin; 24 (74%) were males. The mean age at presentation was 8 years ± 3.3 (range 2 to 16). The average family size was 7 ± 3 family members (range 2 to 13). None of the patients had a history of any other major illnesses. Three (10%) were found to be hypertensive and 12 (39%) had hypocomplementemia (low C3). Eight (26%) had an asymptomatic rise in the liver enzymes (alanine and aspartate amino transferase and gamma glutamyl transpeptidase). None had evidence of acute hepatitis or chronic liver disease. All except one had normal renal function. All had a protein/creatinine ratio in the severe range.

Classification of index cases into categories of hepatitis B virus status

One (3%) of the index cases was classified as category A, and 30 (97%) were classified as category B. However, in three of the index cases in category B, SBH and PCR were not done.

Family members and household contacts (subjects)

One hundred and forty-five (74%) of the 195 family members and household contacts (subjects) were of rural origin; 97 (50%) were males. One hundred and fifty-two (78%) were family members, and 43 (22%) were household contacts. The mean age at testing was 20.3 years ± 15.4 (range 0.5 to 71.2). Twenty-three (12%) had hypertension. One had well-controlled non-insulin–dependent diabetes mellitus, and one had a past history of tuberculosis. Twenty (10%) had an asymptomatic rise in the liver enzymes; none had evidence of acute hepatitis or chronic liver disease. All had normal renal function, as assessed by blood urea and serum creatinine levels and estimation of the glomerular filtration rate using the creatinine transformation formula.

Classification of family members and household contacts (subjects) by hepatitis B virus status

Twelve (6%) subjects were classified as category A, 33 (17%) as category B, 27 (14%) as category C, 36 (18%) as category D, 68 (35%) as category E, and 19 (10%) as category F. Therefore, 72 (37%) subjects were HBV carriers (categories A, B, and C). Thirty-six (18%) were HBV exposed (category D). Sixty-eight (35%) were unexposed (category E), and 19 (10%) were indeterminate for their HBV status (category F; Table 2). Twenty-nine (93%) subjects in category B were HBV DNA positive, giving a total of 56 (29%) subjects positive for HBV DNA. Two (6%) subjects in category B did not have SBH or PCR done.

Hepatitis B virus carriers (categories A, B, and C)

The mean age was 20.1 (SD ± 15) years (range 2.5 to 60.0). Thirty-eight (53%) were males. Sixty-four were family members, and eight were household contacts; these accounted for 42% of all family members and 19% of all household contacts ($P = 0.01$).

Hepatitis B virus exposed (categories C and D)

The mean age was 24.7 (SD ± 17%) years (range 3 to 71). Nineteen (53%) were males. Twenty-five were family members, and 11 were household contacts; these represented 16% of all family members and 26% of all household contacts ($P = 0.25$).

Hepatitis B virus unexposed (category E)

The mean age was 17.1 (SD ± 14) years (range 2 to 50). Thirty-one (46%) were males. Forty five were family members, and 23 were household contacts. These were 30% of all family members and 54% of all household contacts ($P = 0.006$).

Hepatitis B virus indeterminate (category F)

The mean age was 22 (SD ± 15) years (range 0.5 to 58 years). Nine (47%) were males. Eighteen were family members, and one was a household contact, accounting for 12% of the former and 2% of the latter ($P = 0.08$).

The odds ratios for HBV carrier status compared with HBV exposed status in the family members and household contacts was 3.5 [95% confidence limits (CL), 1.3 to 9.5], and for HBV unexposed, it was 4.1 (95% CL, 1.7 to 9.6), respectively. Family members were more predisposed to being HBV carriers and exposed than household contacts ($P = 0.01$ and $P = 0.01$), respectively.

Urinary findings

Forty-five (23%) subjects had microscopic hematuria (maximum 2+ on dipstix analysis) in addition to proteinuria. Fourteen (31%) were carriers. Eight (18%) were exposed. Twenty (44%) were negative, and three (7%) had an indeterminate status for HBV. None had leukocytes, nitrites, or glucose in the urine on dipstix analysis, and all subjects had a negative urinary culture.

Protein/creatinine ratio

Fifty-three (27%) subjects had abnormal proteinuria (Table 3). Their mean age was 14 years (range 2 to 51). Three of four children of less than two years had a protein/creatinine ratio in the normal range, that is, less than 0.5. Twenty-seven (49%) were males. Thirty-nine were family members, and 14 were household contacts. These accounted for 26% of all family members and 33% of all household contacts. Six (11%) family members with abnormal proteinuria had an asymptomatic rise in the liver enzymes. Five (9%) family members with abnormal proteinuria were hypertensive.

Hepatitis B virus status and abnormal proteinuria

We then analyzed the results to detect any association between the different categories of HBV status and abnormal proteinuria (Table 2). Twenty-one (29%) of the HBV carriers, 5 (14%) HBV exposed, 25 (37%) HBV unexposed, and 2 (10%) HBV indeterminate subjects had abnormal proteinuria. There were no statistically significant differences between HBV carriers compared with HBV exposed ($P = 0.07$), HBV unexposed ($P = 0.44$), and HBV indeterminate subjects ($P = 0.14$).

Family members and household contacts, hepatitis B virus status, and abnormal proteinuria

Analysis of subjects into those who were family members and those who were household contacts showed 39 (26%) of 152 family members and 14 (33%) of 43

Table 2. Classification of 30 subjects (family members, household contacts of index children with HBVMN) according to HBV status (using ELISA, slot-blot hybridization and polymerase chain reaction) and protein/creatinine ratio

		Quantitation of proteinuria by protein/creatinine ratio				
Category of HBV status	*N*	Normal	Mild	Moderate	Severe	Not done
A (Hb_sAg positive only)	12 (6.2%)	8 (66.7%)	3 (25.0%)	1 (8.3%)	Nil	Nil
B (Hb_sA and/or Hb_e and/or SBH and/or PCR positive)	33 (16.9%)	22 (66.7%)	9 (27.3%)	1 (3.0%)	1 (3.0%)	Nil
C (Anti-HBc IgG positive only or all markers negative by ELISA but SBH and/or PCR positive for HBV DNA)	27 (13.8%)	20 (74.1%)	1 (3.7%)	3 (11.1%)	2 (7.4%)	1 (3.7%)
D (Anti-HBc IgG positive with SBH and PCR negative)	36 (18.5%)	29 (80.6%)	2 (5.6%)	3 (8.3%)	Nil	2 (5.6%)
E (All negative)	68 (35.0%)	43 (63.2%)	15 (22.1%)	7 (10.3%)	3 (4.4%)	Nil
F (ELISA negative, SBH +PCR not done)	19 (9.7%)	16 (84.2%)	2 (10.5%)	Nil	Nil	1 (5.3%)
Total	195	138 (70.8%)	32 (16.8%)	15 (7.7%)	6 (3.1%)	4 (2.1%)

Abbreviations are: SBH, slot-blot hybridization; PCR, polymerase chain reaction.

Table 3. Comparison of normal and abnormal proteinuria according to the category of HBV status in family members and household contacts of index children with HBVMN

		Quantitation of proteinuria by protein/creatinine ratio			
Category of HBV status	*N*	Normal	Abnormal	Not done	*P* value
Category A					
FM	11	8 (72.7%)	3 (27.3%)	Nil	0.33
HC	1	Nil	1 (100%)		
Total	12	8 (66.7%)	4 (33.3%)		
Category B					
FM	29	19 (65.5%)	10 (34.5%)		0.9
HC	4	3 (75.0%)	1 (25.0%)	Nil	
Total	33	22 (66.7%)	11 (33.3%)		
Category C					
FM	24	19 (79.2%)	5 (20.8%)	Nil	0.41
HC	3	1 (33.3%)	1 (33.3%)	1 (33.3%)	
Total	27	20 (74.1%)	6 (22.2%)	1 (3.7%)	
Category D					
FM	25	20 (80.0%)	3 (12.0%)	2 (8.0%)	0.9
HC	11	9 (81.8%)	2 (18.2%)	Nil	
Total	36	29 (80.5%)	5 (13.9%)	2 (5.6%)	
Category E					
FM	45	29 (64.4%)	16 (36.6%)		0.85
HC	23	14 (60.9%)	9 (39.1%)	Nil	
Total	68	43 (63.2%)	25 (36.8%)		
Category F					
FM	18	15 (83.8%)	2 (11.1%)	1 (5.6%)	0.90
HC	1	1 (100%)	Nil	Nil	
Total	19	16 (84.2%)	2 (10.5%)	1 (5.3%)	
Grand total	195	138 (70.8%)	53 (27.2%)	4 (2.1%)	

Abbreviations are: FM, family members; HC, household contacts.
P value is a comparison of abnormal proteinuria between family members and household contacts.

household contacts to have abnormal proteinuria (P = 0.48).

These family members and household contacts with abnormal proteinuria were then classified into the various categories according to their HBV status (Table 3). Of the 39 family members with abnormal proteinuria, 18 (46%) were HBV carriers. Three (8%) were HBV exposed. Sixteen (41%) were HBV unexposed, and two (5%) were indeterminate. Of the 14 household contacts with abnormal proteinuria, three (21%) were HBV carriers. Two (14%) were HBV exposed. Nine (64%) were HBV unexposed, and none were indeterminate. Comparison between family members and household contacts with abnormal proteinuria among these who were HBV carriers to those who were HBV exposed (P = 0.24) and HBV unexposed (P = 0.18) showed no statistically significant differences. We next compared the different categories of HBV status according to whether they were family members with abnormal proteinuria or household contacts with abnormal proteinuria (Table 3). There were no statistically significant differences using chi-square analysis.

Gender, hepatitis B virus status, and abnormal proteinuria

We compared gender differences in the various categories of HBV status for normal and abnormal proteinuria. Twenty-seven (51%) of those with abnormal proteinuria were males, and 26 (49%) were females. A comparison of gender differences in subjects with abnormal proteinuria in each of the categories according to HBV status showed no statistically significant differences except in category B, with females having a higher frequency of abnormal proteinuria than males (P = 0.05). In addition, gender had no impact on HBV status (P = 0.44).

Age, hepatitis B virus status, and abnormal proteinuria

We studied the impact of age on proteinuria in subjects in the various categories of HBV status. Children less than five years old had a higher prevalence (53%) of abnormal proteinuria than those older than five years (23%, P = 0.008). Age had no impact on HBV carriage (P = 0.42).

Hepatitis B virus genotype

The precore region of HBV DNA was extracted from 10 index cases and 14 family members and household contacts. All were genotype A, and there were no major differences in this region between index cases and family members and/or household contacts.

Community-based controls

One hundred and twenty-three control subjects had their protein/creatinine ratio done. All were black. Twenty-eight were less than five years old, and 53 (43%) were males. One hundred and five (85%) had a protein/creatinine ratio in the normal range. Thirteen (11%) had mild proteinuria, and five (4%) had moderate proteinuria. Children less than five years old had a higher incidence of abnormal proteinuria (21%) compared with older children and adults (12%) (Table 2), but the difference was not statistically significant (P = 0.22) on a chi-square analysis (Table 4).

Table 4. Incidence of proteinuria in family members and household contacts compared to community based controls

Number	Family members and household contacts	Community based controls	P values
Normal proteinuria			
<5 years	14/31	30/38	0.001
>5 years	122/161	75/85	0.03
Abnormal proteinuria			
<5 years	18/34	8/38	0.01
>5 years	37/161	10/85	0.02

DISCUSSION

The main findings of covert proteinuria and clustering of HBV within families and household contacts in this study are not new if considered individually [23–27]. What is new is the high prevalence rate of asymptomatic proteinuria (28%) in families and household contacts of index children with HBV nephropathy. Indeed, this is the highest prevalence of this abnormality yet reported. The most intriguing observation, however, is the absence of any correlation among family members and household contacts between HBV markers and asymptomatic proteinuria. The dissociation between HBV carriage and proteinuria is further highlighted by the fact that although proteinuria was most frequent in children under five years, there were no significant differences in HBV carriage in either age range. These findings support a conclusion that is the converse of the hypothesis we set out to test. In the light of these unexpected results, we need to find a different explanation for the biosocial context and pathogenesis of HBVMN.

Although it is difficult to determine the exact source of infection in these children with HBVMN, it is possible that it was acquired from members within the households, given the high level of clustering. It is possible that there are certain risk factors, behaviors, or exposures within families and household contacts of children with HBVMN that predispose them to the intensity of clustering of HBV in these households. As these households were from a lower socioeconomic group with inadequate facilities, this resulted in overcrowding and close contact between occupants. Risk behaviors such as sharing of household items, for example, toothbrushes, razors, and

wash cloths, are prevalent in these household and may contribute to mechanisms of HBV transmission [28].

In a previous study of clustering of HBV in the home backgrounds of children identified as HBV carriers and HBV exposed and HBV unexposed individuals, we detected prevalences of HBV carriage of 20, 9, and 3% in the families and household contacts of these three groups, respectively [27]. In this study of children with HBVMN, we show that 17% were both HB_sAg and HB_eAg positive with or without HBV DNA; 6.2% had only Hb_sAg, and 13.8% were anti-HBc IgG positive or ELISA negative but SBH or PCR positive, that is, a total of 37% HBV carriage. Thus, the figures for HB_sAg (with or without HB_eAg) carriage in families and household contacts of the index Hb_sAg carrier children in the earlier study and the index children with HBVMN in this study are not significantly different. The population group studied is the same, but there is a 12-year interval between these reports. The HBV vaccination given in the Extended Programme for Immunization (EPI) was introduced into South Africa in April 1995 and could not have influenced these findings.

The techniques employed to detect HBV and proteinuria require comment. The two methods used to detect HBV DNA (SBH and PCR) enabled us to identify 15 family members and household contacts with HB_sAg only who had active viral replication (category B), 12 children who were negative for HB_sAg, HB_eAg, and anti-HBc IgG but SBH or PCR positive, and a further 15 children positive for anti-HBc IgG but SBH and/or PCR positive (category C). Measurement of the protein/creatinine ratio in single urine samples is potentially more accurate than 24-hour urine collection because it avoids collection errors and may give more physiologically relevant information [2]. Thus, in the family members and household contacts of patients with HBVMN, we used this noninvasive method to uncover subtle forms of underlying renal dysfunction. Renal biopsies would have been informative but are self-evidently unethical in these asymptomatic subjects. This is the first time, to our knowledge, that a sensitive method such as the protein/creatinine ratio has been used to detect subtle forms of renal damage, in contrast to other epidemiological studies of asymptomatic proteinuria [23–27].

Abnormal proteinuria that is independent of HBV status in family members and household contacts is the most striking observation in this study and forms the core of the hypothesis we advance to explain our results. Central to our hypothesis is that the amounts of protein excreted in the urine of these asymptomatic subjects exceed the normal threshold and are surrogate manifestations of an underlying glomerular basement membrane disorder. Proteinuria is one of the main expressions of kidney disease [29]. Although proteinuria can occur as a result of glomerular or tubular disorders, proteinuria detected by urinary dipstix analysis is usually indicative of disturbances in glomerular filtration [30]. Physiological causes of proteinuria such as exercise, fever, and high altitudes were excluded in all family members and household contacts. Durban is a seaport, and families were mostly from the coastal regions. The possibility remains that some of these family members and household contacts may have had orthostatic proteinuria. We believe that this is unlikely in the majority, however, because protein excretion rates rarely exceed 1 g per 24 hours in orthostatic proteinuria [31–33], and this level was detected in 70% of the family members and household contacts with proteinuria in this study. Manifestations of renal disease and renal insufficiency were not evident in any of the family members or household contacts. A number of studies have recorded the prevalence of asymptomatic proteinuria in children and adults, but prevalences were much lower than that reported here. In a large study, isolated proteinuria was detected in 2.5% of children aged 8 to 15 years [34]; in adults, the incidence of isolated proteinuria in the absence of underlying renal disease varies from 0.4 to 5% in different studies [35]. Reports from the United States in children with similar proteinuria suggest that in the long term, although the majority of children with urinary abnormalities have either no renal disease or at most a self-limited condition, a small percentage (2%) have some form of underlying renal pathology [23]. A similar study in adults in Japan showed that approximately 10% of subjects with asymptomatic hematuria and/or proteinuria followed up for long periods had various forms of renal pathology [34]. Unfortunately, there are no comparable prevalence studies of microalbuminuria or determination of long-term outcome in the population group we studied.

The high prevalence of proteinuria in the African households studied and in community controls may be the result of exposure to common environmental antigens (helminths, bacteria, viruses, toxins, and drugs) that produce mild degrees of glomerular basement membrane damage [2]. This is a well-recognized association. A recent hypothesis on the pathogenesis of MN suggests a predisposition in different individuals for vigorous IgG_4 responses to certain antigens (helminths, drugs, etc.), which may be genetic in nature [35]. It is possible that these factors may account for the increased proteinuria in household contacts, although we did not specifically screen for these agents. HBV carriage and abnormal proteinuria rates were not significantly different between family members and household contacts. Accordingly, there does not appear to be a heritable component to the development of either of these two disorders. This finding corroborates the results of our earlier study [27] in which HBV carriage was unrelated to family membership.

Associations between different loci on the HLA com-

plex and susceptibility or resistance to the chronic HBV carrier state, chronic active hepatitis, and HBVMN have been reported, although the findings are not conclusive [6]. In a study of black children with NS from our region, an association of MN with HLA Bw21 was detected [36]. The HLA D locus, however, was not studied in this group. We have done HLA studies in the index cases with HBVMN, as well the family members and household contacts, which will form the basis of another report. This evidence on genetic factors influencing outcome of HBV infection is uneven. Although some studies have shown a genetic predisposition to the development of HBV carriage [37, 38], other studies have refuted a genetic predisposition to the HBV carrier state [39–41]. Thus, on the balance, there appears to be a complex interplay of heritable traits together with environmental factors and certain high-risk behaviors that render these children open to HBV carriage.

In brief, this article, which portrays one aspect of the biosocial context of HBVMN, describes a concentration of individuals with high prevalence of the HBV carrier state and abnormal proteinuria within the families and household contacts of children with HBVMN. We argue that this combination of findings implies an environmental or social vulnerability in the family members and household contacts of children with HBVMN to multiple antigen exposure, including HBV. Among young children, exposure to multiple antigens within these households leads to glomerular dysfunction. Within households of the index cases of HBVMN, there is substantial further risk of HBV carriage. The operation of additional factors, especially genetic influences, on children within these settings may produce HBVMN. Given the unexpected outcome of our study, it is plausible that HBV infection is associated with HBV-induced MN, but not causally related to it.

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