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Hepatitis B surface antigen in urine of hemodialysis patients

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Hepatitis B surface antigen in urine of hemodialysis patients. As part of an extensive epidemiological survey of chronic hemodialysis patients in Michigan, hepatitis B surface antigen (HB_sAg) was identified in the sera of 79 of 701 (11%) patients. Of these patients, 59 were carriers of HB_sAg for three or more months. Urine samples were collected from 36 of 39 HB_sAg carriers having urinary output. Of these samples, 19 (52%) were positive for HB_sAg by radioimmunoassay; this was confirmed by specific antibody neutralization. The HB_sAg was not identified in the urine of seven hemodialysis patients who were lacking serum HB_sAg or in urine samples from three HB_sAg sero-carriers who had normal renal function. Patients undergoing maintenance hemodialysis appear to constitute a large reservoir of HB_sAg chronic carriers. This study indicates that a minimum of 50% of persistent HB_sAg carriers who are producing urine have detectable HB_sAg in single, randomly timed, unconcentrated urine specimen. These data suggest that urine may represent a potential vehicle for transmission in nonparenterally acquired hepatitis B.

Antigène de surface de l'hépatite B dans l'urine de malades en hémodialyse. Dans le cadre d'une large enquête épidémiologique à propos des malades en hémodialyse chronique dans le Michigan, l'antigène de surface de l'hépatite B (HB_sAg) a été identifié dans le sérum de 79 parmi 701 malades (11%). Parmi ces malades, 59 étaient des porteurs de HB_sAg depuis 3 mois ou plus. L'urine de 36 des 39 porteurs de HB_sAg, qui avaient une diurèse, a été recueillie. Parmi ces 36 urines, 19 (52%) sont positives pour HB_sAg par radio-immunologie, ce qui est confirmé par la neutralisation au moyen d'anticorps spécifique. Le HB_sAg n'a pas été identifié dans l'urine de 7 malades en hémodialyse qui n'avaient pas le HB_sAg sérique et dans l'urine de 3 porteurs de HB_sAg dont les fonctions rénales étaient normales. Les malades soumis à l'hémodialyse itérative paraissent constituer un grand réservoir de porteurs chroniques de HB_sAg. Cette étude indique qu'au minimum 50% des porteurs chroniques de HB_sAg qui ont une diurèse, ont un HB_sAg détectable dans un échantillon unique d'urine, prélevé au hasard, non concentré. Ces résultats suggèrent que l'urine peut être un véhicule de transmission de l'hépatite B acquise par voie non parentérale.

Hepatitis B is an important cause of morbidity and mortality among patients with end-stage renal disease. Of additional importance is the risk of infection for persons having intimate contact with these patients and/or their secretions. Previous studies [1–8] have estimated that the prevalence of hepatitis B infection varies from 0 to 100% among dialysis patients and up to 40% among hospital personnel dealing with these patients. Furthermore, up to 61% of the family members of dialysis patients with a history of hepatitis B were found to have hepatitis B surface antigen (HB_sAg) or hepatitis B surface antibody (anti-HB_s) in their sera [2]. Previous studies have demonstrated HB_sAg in saliva [9–11], semen [9], and breast milk [12], suggesting transmission by these secretions. Other studies have documented that HB_sAg is present in menstrual blood [13] and in vaginal secretions [14] of female antigen carriers. While the exact mode(s) of transmission of hepatitis B in hemodialysis units remains unclear, previous epidemiologic studies [2, 3, 15] have neglected the possible role of urine as a vehicle for the spread of hepatitis B. Studies [11, 16–22] that have examined urine as a transmission vehicle involved subjects with virtually intact glomeruli and no proteinuria. Presence of hepatitis antigen in the urine of each of seven renal transplant recipients has been reported [23]. Because of the impaired glomerular permeability of patients who have end-stage renal disease, and since many of these patients produce significant amounts of urine, this study was undertaken to examine a large population of these patients to determine the incidence of HB_sAg-positive urine.

Methods

This study is part of an epidemiologic survey of dialysis patients which involves 27 of 29 hemodialysis units in Michigan. In this survey, 701 patients of a possible 800 on center chronic hemodialysis were studied. There were 59 patients identified as persis-

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tent carriers of HB_sAg for a period ranging between three months and three years. An additional 20 patients were discovered to be antigenemic during the initial survey sampling and are not included in this study.

Urine specimens were obtained from 36 of the 59 persistent HB_sAg carriers. Of the remaining 23 persistent carriers, 13 were anephric, 7 had no urinary output, and in 3 cases cooperation in obtaining specimens was lacking. In addition, 7 urine specimens were obtained from hemodialysis patients who were HB_sAg-negative, and 3 urine specimens were obtained from healthy carriers of serum HB_sAg. All urine samples were coded and stored at −20°C. Sample volumes ranged from 5 to 200 ml, and they were not concentrated prior to testing.

All urine specimens were tested for HB_sAg by radioimmunoassay (Ausria II-125, Abbott Laboratories). Two-tenths of a milliliter of unconcentrated urine was incubated in wells for 16 hr at 20 to 23°C (room temperature) with anti-HB_s-coated beads. After incubation, the beads were washed three times with 5 ml of glass distilled water, and ^{125}I-labeled anti-HB_s was added to each well. The samples were then incubated for 60 min in a 45°C water bath, and the beads were rinsed, as described above. The residual radioactivity on each bead was counted immediately in a gamma-counter for 60 sec. The net negative control mean value was calculated from data obtained from seven beads that reacted with a single-source urine sample from an HB_sAg negative individual. The urine samples with 2.1 times the net negative control value were considered as presumptive positives for HB_sAg.

Urine specimens positive for HB_sAg by the above method were confirmed using the specific antibody Confirmatory Neutralization Test (Ausria II-125, Abbott Laboratories) rather than other licensed but less sensitive HB_sAg test systems. Initial incubation and washing of antibody-coated beads with unconcentrated urine was performed as above. Human anti-HB_s was added then to the wells for neutralization of bound patient HB_sAg during an incubation at 45°C for one hour. ^{125}I-anti-HB_s was subsequently added to the wells without washing. After incubation at 45°C for three hours, the contents of the wells were aspirated, washed, and transferred to tubes for gamma scintillation spectrometry. The net negative control mean was calculated from data obtained from seven beads incubated with urine from an HB_sAg non-reactive individual. A presumptive positive sample was considered a confirmed positive for HB_sAg if 50% or more of the radioactivity was inhibited by specific antibody.

All urine samples were tested for the presence of protein and occult blood by Bili-Labstix (Ames Company).

Results

Of the 36 urine samples collected from persistent serum HB_sAg carriers, 20 (55%) were positive for HB_sAg by radioimmunoassay. Nineteen of these positive urines were confirmed by specific neutralization, and suppression of radioactivity by neutralizing antibody was usually much greater than 50%. Only one initially reactive urine specimen could not be confirmed.

Figure 1 shows the distribution of corrected counts per minute from urine samples of both HB_sAg sero-positive and sero-negative end-stage renal disease patients. In addition, the counts per minute from

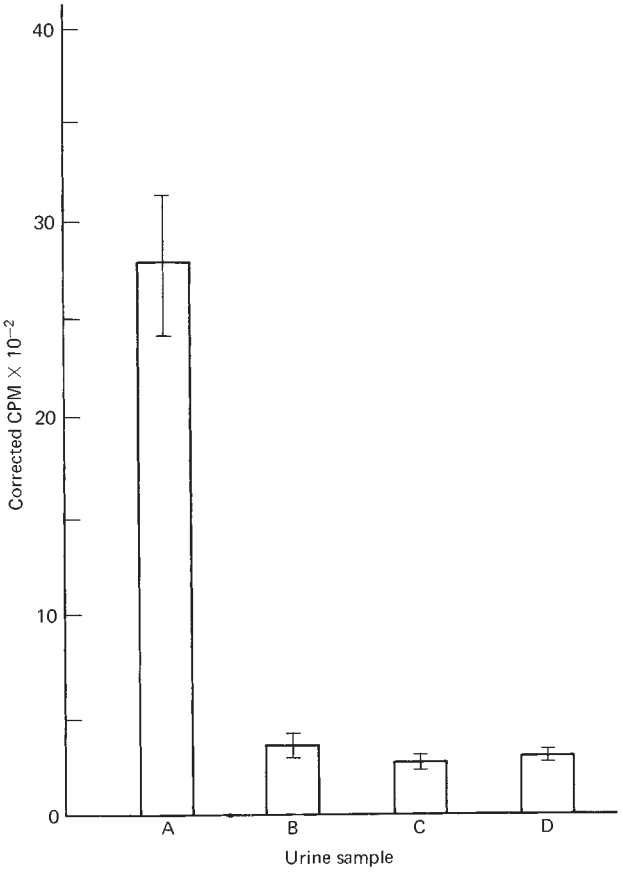

Fig. 1. *Corrected counts per minute of ^{125}I-anti-HB_s bound in urine samples from: A, persistent HB_sAg sero-positive, end-stage renal disease patients with HB_sAg in urine (mean of 19 patients); B, persistent HB_sAg sero-positive end-stage renal disease patients without HB_sAg in urine (mean of 17 patients); C, HB_sAg sero-negative end-stage renal disease patients (mean of 7 patients); and D, healthy sero-carriers of HB_sAg without renal disease (mean of 3 patients).* The *bracketed lines* indicate ±1 SEM.

Table 1. Urine HB_sAg and occult blood in persistent sero-positive HB_sAg in patients with end-stage renal disease

Urine occult blood		Urine HB_sAg	
		Positive	Negative
Positive	5	2	3
Negative	31	17	14
Total	36	19	17

urine samples of three sero-positive persons without renal disease are included. HB_sAg was not found in any of the seven urine specimens from dialysis patients without detectable serum HB_sAg, nor in three urine specimens from healthy individuals who are carriers of HB_sAg in their sera.

As shown in Table 1, occult blood was found in five urine specimens of the 36 samples obtained from dialysis patient carriers of HB_sAg, but only two of these were found to contain HB_sAg. None of the urine samples from seven sero-negative end-stage renal disease patients or three healthy carriers showed the presence of occult blood. All 36 specimens collected from patients having end-stage renal disease exhibited proteinuria, but none of the urine samples from three healthy carriers exhibited proteinuria.

Among sero-positive hemodialysis patients, serum transaminase activities, bilirubin levels, age, sex, duration of hemodialysis, duration of HB_sAg carrier state, volume of urinary output and the original nature of end-stage renal disease were not significantly different by analysis of variance ($P > 0.05$) in patients with or without HB_sAg in their urine.

Discussion

Using a solid-phase radioimmunoassay to detect HB_sAg in unconcentrated urine samples, we have demonstrated HB_sAg in 52% of the urine specimens from hemodialysis patients who are persistent carriers of HB_sAg in their sera. This finding is not related to the presence of gross or occult blood in the urine. Verification of presumptive HB_sAg positive urine samples was accomplished using a specific human anti-HB_s neutralization assay.

There are conflicting reports regarding the presence of HB_sAg in urine. Ogra [18] has shown that viruria is detectable in a small number of hepatitis patients for a short time in comparison to fecal shedding of virus. In their study of seven renal transplant recipients who had become chronic serum carriers of hepatitis antigen, Blainey et al [23], using a complement fixation test, detected low levels of antigen in the unconcentrated urine of each patient. The presence of hepatitis antigen in the urine samples from these patients may be linked to residual output from their original kidney(s). Apostolov et al [19] have reported Australia antigen in the urine from 8 of 13 sero-positive patients with acute viral hepatitis, and in 4 of 13 sero-positive patients with chronic liver disease; however, they also reported Australia antigen in the urine in 4 of 13 sero-negative patients with chronic liver disease, and in 2 of 7 healthy volunteers who were not carriers of Australia antigen in their serum. In addition, all their HB_sAg positive urines were detected by the counterimmunoelectrophoresis assay after 70- to 80-fold concentration of the urine samples. Also, the antibody used in their study was obtained from one of the HB_sAg sero-negative patients who had biliary cirrhosis and who was not fully characterized.

Heathcote, Tsianides, and Sherlock [17] have reported that 10% of urine samples collected from 52 patients with acute viral hepatitis were positive for hepatitis B antigen by the complement fixation technique. Of the total urines, 62% were positive on at least one occasion during six months of continued follow-up study. These authors, however, found that 48% of the household contacts of these patients had detectable antigen in their urine without being carriers of hepatitis B antigen in their serum.

In contrast to the results of Blainey et al [23], Apostolov et al [19], and Heathcote et al [17], other reports [11, 21] have shown that only 3 of 130 urine samples from sero-positive carriers (each of the 3 having occult blood in the urine at the time of testing) and 1 of 13 urine specimens from HB_sAg sero-positive blood donors were positive for HB_sAg by radioimmunoassay. Furthermore, no HB_sAg was detected in the urine of patients with acute or chronic hepatitis [21] or of blood donor volunteers with serum HB_sAg [22]. Finally, Irwin et al [16], using a radioimmunoassay, did not detect HB_sAg in unconcentrated urine samples of 43 HB_sAg sero-carriers. A 100-fold concentration of urine samples from these patients, however, resulted in seven patients (16%) showing detectable HB_sAg.

Our findings indicate that the prevalence of HB_sAg in urine of end-stage renal disease patients with HB_sAg positive serum is substantial. Physiological mechanisms that might account for this finding, however, remain unclear. We speculate that altered glomerular premeability could account for the substantial number of patients with detectable urine HB_sAg. This hypothesis is supported by the finding of qualitative proteinuria in all end-stage renal disease patients in our study and those of Irwin [16] demonstrating urine HB_sAg concentrations 100-fold greater

than serum in patients with abnormal renal function and proteinuria. In addition, none of our three healthy carriers (without proteinuria) have HB_sAg-positive urine, and only a small percentage of patients from previous studies in whom renal function was presumably not compromised have HB_sAg-positive urine.

No significant difference ($P > 0.05$) in urine output of the group having HB_sAg in their urine, vs. those without, rules out the possibility that HB_sAg-negative urine patients had a large volume of dilute urine resulting in levels of HB_sAg below the limits of detection by the radioimmunoassay. In fact, several patients with urine which was positive for HB_sAg had a urinary output in excess of 1,000 ml per day. It would be of interest, however, to monitor those patients who are negative for HB_sAg in their urine, following concentration of the urine specimens and repeated urine sampling to maximize detection of viruria.

Hepatitis B infection among hemodialysis patients is an endemic problem of relatively high frequency. At the time of this survey, 90% of the 27 hemodialysis units studied reported hepatitis B infections. Approximately 25,000 patients are being dialyzed throughout the United States. From the carrier data in our study, at least 2,750 patients might be expected to be HB_sAg carriers at any given time, 1,900 would have significant urine output, and 1,000 patients would have detectable HB_sAg in the urine.

HB_sAg in the urine may not correlate with its infectivity. Further evidence is needed to confirm the infectivity of urine and to define the characteristics of infectious urine, particularly to determine if all morphological forms of HB_sAg, including the whole virions (Dane particles), cross the glomerulus. Recent studies [24–26] have suggested that the presence of DNA polymerase and e antigen in serum of HB_sAg-positive patients appear to be indicators of the relative infectivity of this serum. This is particularly true in non-percutaneous transmitted hepatitis after small-volume exposure. To our knowledge, there is no available evidence regarding the presence of e antigen or DNA polymerase in urine that is positive for HB_sAg. There is, however, some evidence suggesting that these two apparent indices of infectivity are not found in the absence of HB_sAg in the serum [26]. Thus, it is probable that all urines positive for HB_sAg are not equally infectious and that some are not infectious at all. The ability of e antigen and DNA polymerase to define the relative infectivity of HB_sAg-positive blood may also apply to HB_sAg-positive urine. Until it is certain that all body fluids, including urine, negative for e antigen and polymerase are non-infectious, and also until infectivity tests are available to all dialysis centers, urine from antigenemic hemodialysis patients should be considered a potential vehicle for the transmission of hepatitis B virus.

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