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Hepatitis B Antigen in Saliva, Urine, and Stool

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A survey of hepatitis B patients, asymptomatic hepatitis B antigen (HB_sAg) carriers, and control subjects was conducted to determine the relationship between antigenemia and antigen excretion in saliva, urine, and stool. Radioimmunoassay was used to detect HB_sAg. Specificity-confirmed HB_sAg was detected in the saliva of 6 (30%) of 20 antigenemic patients, 1 (5%) of 20 nonantigenemic patients, 14 (34%) of 41 carriers, and 0 of 112 controls. HB_sAg was detected in urine only after 100-fold concentration of first-morning specimens. Specificity-confirmed HB_sAg was present in the urine of 7 (16%) of 43 carriers; unconfirmed HB_sAg was found in the urine of 5 (13%) of 38 patients and 5 (5%) of 112 controls. Unconfirmed HB_sAg was found in the urine of 5 (13%) of 38 patients and 5 (5%) of 112 controls. Unconfirmed HB_sAg was detected in concentrated stool specimens from 5 (46%) of 11 patients and 0 of 8 carriers and controls. Longitudinally collected specimens from antigenemic subjects showed no consistent patterns of antigen excretion.

Asymptomatic hepatitis B antigen (HB_sAg) has been detected in serum (15), urine (2, 14), stool (3, 8), seminal fluid (4), and menstrual blood (8). Since HB_sAg-positive serum has been shown to be capable of transmitting hepatitis B infection by mouth (6), the presence of HB_sAg in the other substances implies the potential for nonparenteral transmission of hepatitis B virus (HBV). This survey was conducted to determine the relationship between antigenemia and excretion of HB_sAg in saliva, urine, and stool in acute hepatitis cases and asymptomatic antigen carriers.

MATERIALS AND METHODS

Subjects. The following populations were studied: hepatitis patients at Fort Hood, Tex. and at Walter Reed Hospital, Washington, D.C.; orthopedic patients at Fort Hood (controls); healthy, nonhospitalized military personnel at Fort Hood and at the Walter Reed Army Institute of Research, Washington, D.C. (controls); and HB_sAg carriers at Fort Hood. All of the latter had normal liver function tests and persistent antigenemia for at least 3 months. The antigen-negative hepatitis patients included in the study were believed to have hepatitis B infections because of antibody seroconversion and exposures to HB_sAg-positive people.

Specimen collection. From 5 to 10 ml of saliva and 100 ml of first-morning urine were collected twice weekly from hepatitis and orthopedic patients during their hospital stay. A stool specimen was obtained once a week. Saliva and urine samples were collected in sterile glass jars and stored at -20 C until tested for HB_sAg. In six hepatitis patients, 24-h urine collections were obtained. Blood was drawn from all subjects, and the serum was stored at -20 C. Convalescent samples of serum, urine, and saliva were obtained from all available hepatitis patients 3 months after hospital admission. Reagent strips (Bili-Labstix, Ames Co.) were used to detect blood in saliva and urine. Reagent tablets (Hematest, Ames Co.) were used for detecting blood in stool.

Concentration of urine and stool. Because HB_sAg was not found in any unconcentrated urine samples, first-morning urine specimens (100 ml) were concentrated 100-fold by filtration across PM-30 membranes (Amicon). After the concentration procedure, the supernate as well as a 1 to 2 ml of eluate of the PM-30 membrane were stored at -20 C until tested for HB_sAg. Urine samples (24 h) were concentrated 250- to 500-fold over a single membrane and processed as above.

Stool samples (20 g) were homogenized in 100 ml of tris(hydroxymethyl)aminomethane buffer and then concentrated 100-fold as above. Portions (0.2 ml) of supernatant and eluate were adjusted to pH 2.0 with HCl because preliminary tests (using stool specimens to which HB_sAg-positive serum had been added) showed that this procedure would increase the rate of detection of HB_sAg in stool. Both acid-treated and untreated, concentrated stool samples were tested for HB_sAg.

HB_sAg testing. Portions (0.1 ml) of concentrated urine and stool specimens, and unconcentrated saliva and serum samples, were processed as described for serum by radioimmunoassay (RIA) using the Ausria test kit (7). Saliva was not concentrated because of limited quantities. HB_sAg-positive controls of saliva, urine, and stool were prepared by serially diluting

counterelectrophoresis-positive sera in saliva, urine, and stool from normal, nonantigenemic people. Tenfold dilutions, from 10^{-1} to 10^{-6}, were made, and dilution curves were plotted. For each test run, both dilution-curve samples and 10 replicates of an HB_sAg-negative control of saliva, concentrated urine, or stool were included. A specimen yielding counts per minute greater than 5 standard deviations above the mean of the 10 replicates of the appropriate control was considered positive. Because false-positive reactions have occurred with the RIA test (1, 5, 13), RIA-positive sera were tested for specificity by an inhibition test using antibody (anti-HB_s) to surface antigen.

RESULTS

The frequency of detection of HB_sAg in saliva from patients, carriers, and controls is shown in Table 1. There was no apparent trend in rates of positivity of specimens collected at twice weekly intervals from patients. The nonantigenemic hepatitis patient with antigen in his saliva had anti-HB_s in his convalescent serum, indicating HBV infection. The presence of small amounts of blood in the saliva specimens did not appear to influence the rate of detection of HB_sAg, since the proportion of specimens containing blood (60%) was the same in both antigen-positive and antigen-negative specimens.

The results of testing concentrated first-morning urine specimens for HB_sAg are presented in Table 2. Eleven (18%) of 63 antigenemic subjects had HB_sAg-positive urine. Because of low counts per minute specificity was confirmed on only 7 (27%) of the 26 positive samples from these subjects. When 24-h urine collections from three randomly selected antigenemic hepatitis patients were concentrated 500-fold, two patients had no detectable antigen in their urine, whereas the third had specificity-confirmed HB_sAg in each of five samples.

The data from asymptomatic carriers were examined for a possible association between antigen in urine and antigen in saliva: only 3 of the 41 subjects tested had antigen in both, and there was no statistically significant association ($P > 0.05$, Fisher exact test).

Blood was not detected in any urine samples. All antigenemic persons with detectable HB_sAg in their urine had normal blood urea nitrogen levels.

Tests for HB_sAg were conducted on stool samples from 19 subjects (Table 3). None of the positive tests could be confirmed due to low counts per minute.

A comparison was made between the dilution-curve counte per minute of HB_sAg-positive control specimens (prepared as described above) and counts per minute of saliva, urine, and stool samples from antigenemic patients. Counts per minute of unconcentrated saliva from patients were similar to those of the controls, in a range of 10^{-3} to 10^{-4}, but counts per minute of urine and stool samples were equivalent to 10^{-3} and 10^{-4} only after 100 to 500 $\times$ concentration.

The patterns of detection of HB_sAg in serially collected specimens of saliva, urine, and stool from three representative antigenemic subjects are shown in Table 4.

Serum, saliva, and urine specimens were collected from 11 antigenemic and 9 nonantigenemic hepatitis patients 3 months after the onset of illness. HB_sAg was detected in the serum of one antigenemic patient and in the saliva of another; all other specimens from both groups of patients had no detectable HB_sAg. Six of the nine nonantigenemic patients had anti-HB_s in their convalescent sera.

DISCUSSION

HB_sAg has been found in serum in three morphologic forms: most commonly as a sphere

TABLE 1. *Detection of HB_sAg in unconcentrated saliva*

Subjects	Serum HB_sAg[a]	HB_sAg in saliva			
		Subjects		Samples	
		No. positive/no. tested	Percent	No. positive/no. tested	Percent
Hepatitis patients	Pos.	6/20	30	13[b]/86	15
	Neg.	1/20	5	1/68	2
Asymptomatic carriers	Pos.	14/41	34	14/41	34
Orthopedic patients	Neg.	0/35	0	0/35	0
Healthy adults	Neg.	0/77	0	0/77	0

[a] Pos., Positive; Neg., negative.
[b] Two of 13 specimens unconfirmed.

TABLE 2. *Detection of HB_sAg in concentrated samples of first-morning urine*

Subjects	Serum HB_sAg[a]	HB_sAg in urine			
		Subjects		Samples	
		No. positive/no. tested	Percent	No. positive/no. tested	Percent
Hepatitis patients	Pos.	4/20	20	6/73	8
	Neg.	1/18	6	1/65	2
Asymptomatic carriers	Pos.	7[b]/43	16	19[b]/45	42
Orthopedic patients	Neg.	5/35	14	5/70	7
Healthy adults	Neg.	0/67	0	0/67	0

[a] Pos., Positive; Neg., negative.
[b] Six of 7 subjects and 7 of 19 samples had HB_sAg confirmed by specificity tests; none of the rest was confirmed.

22 nm in diameter; as a filamentous structure 22 nm in cross-section; and as the outer surface of the 45-nm Dane particle, which is believed to be the HBV. In serum from patients and carriers, the number of 22-nm particles greatly exceeds the number of Dane particles (i.e., presumably infectious virions). Whether a similar ratio of particles exists in saliva, urine, and stool is unknown but may be important in terms of transmission of HBV. For example, if most of the HB_sAg is in the form of Dane particles, then the substance is more likely to be infectious than if most is in the form of 22-nm particles. Therefore, the full implications of detecting HB_sAg in saliva, urine, or stool are as yet unknown; however, epidemiological studies of close personal contacts of asymptomatic HB_sAg carriers strongly support the concept of nonparenteral transmission of HBV (5, 13).

Although HB_sAg was most frequently found in saliva, it is likely that an even higher proportion of patients and carriers would have been found to excrete HB_sAg in saliva had concentration of specimens been possible. Among subjects with detectable HB_sAg in saliva and urine, we estimate the concentration of antigen in saliva to be 1,000-fold less than that in serum and 100-fold greater than that in urine. The sporadic detection of HB_sAg in saliva appears to be a quantitative phenomenon, reflecting the concentration of antigen in serum as measured by RIA.

This study confirmed the high frequency of blood in saliva previously reported by Ward et al. (15). However, no correlation between presence of blood and detection of antigen was observed. Since there are no reported data indicating that HB_sAg replicates on mucosal surfaces, the mechanism by which HB_sAg appears in saliva in the absence of blood remains unexplained.

In this as in previous studies (14, 2, 8), HB_sAg was detected in urine only after concentration of relatively large volumes (100 to 500 ml). However, previously employed detection techniques differed from those we used, and included affinity chromatography absorption methods (14), dialysis-lyophilization (8), and dialysis-ultracentrifugation pelleting procedures (4). All of the concentrated urine specimens obtained from antigenemic patients and carriers in this study yielded low counts per minute, and therefore the specificity for HB_sAg of an RIA-positive sample could not routinely be confirmed. The occurrence of positive urine tests in five or-

TABLE 3. *Detection of HB_sAg in stool*

Subjects	Serum HB_sAg[b]	HB_sAg in stool[a]	
		Subjects	Samples
		No. positive/no. tested	No. positive/no. tested
Hepatitis patients	Pos.	2/7	2/11
	Neg.	3/4	3/5
Asymptomatic carriers	Pos.	0/3	0/3
Healthy people	Neg.	0/5	0/5

[a] Specificity not confirmed.
[b] Pos., Positive; Neg., negative.

TABLE 4. *Detection of HB_sAg in serial samples of saliva, urine, and stool from three antigenemic subjects*

Subject	Sample	Presence of HB_sAg[a]							
		0[b]	5	10	20	30	45	60	90
Subject 1 (hepatitis patient)	Serum HB_sAg	+	+	+	+	+	+	+	ND
	Saliva HB_sAg	+	+	−	+[c]	+	−	+[c]	ND
	Urine HB_sAg	−	−	−	−	−	−	−	ND
	Stool HB_sAg	ND	−	ND	−	ND	ND	ND	ND
Subject 2 (hepatitis patient)	Serum HB_sAg	+	+	+	+				−
	Saliva HB_sAg	−	−	−	−				−
	Urine HB_sAg	+	+	−	−				−
	Stool HB_sAg	ND	+[d]	−	ND				ND
Subject 3 (asymptomatic carrier)	Serum HB_sAg	+	+	+	+				+
	Saliva HB_sAg	−	ND	+	+				−
	Urine HB_sAg	+	−	ND	+				+
	Stool HB_sAg	ND	ND	ND	ND				ND

[a] +, HB_sAg detected; −, HB_sAg not detected; ND, not done. Blank spaces indicate no samples were obtained.
[b] Number of days after infection.
[c] Blood detected in saliva.
[d] Specificity for Hb_sAg not confirmed.

thopedic patients (controls) showed that false-positive reactions were likely to occur and demonstrated the need for testing to confirm specificity. Comparisons between counts obtained from urine and from serially diluted, counterelectrophoresis-positive sera suggested that the concentration of HB_sAg in the urine of antigenemic patients and carriers was 10^{-5} to 10^{-7} times that in their serum. High concentrations of HB_sAg (10^{-2} times that in serum) may be present in the urine of persons with abnormal renal function and proteinuria, as demonstrated by three patients studied by us but not reported in this survey.

Stool specimens from hepatitis patients yielded low counts per minute, and therefore specificity for HB_sAg could not be established; consequently, we could not confirm the results reported by Grob and Jemelka (3). Possible explanations for the difference in results include degradation of HB_sAg prior to testing; excretion of HB_sAg in stool late in the course of the illness; differences in patterns of HB_sAg excretion in stool among patients infected by the gastrointestinal route as compared to those infected by the parenteral route; and action of an intestinal inhibitor of HB_sAg, as described by Piazza et al. (10).

Our findings suggest that when HB_sAg is present in the serum, it may also be in the saliva, urine, and stool. Whether the presence of HB_sAg in these substances will correlate with their infectivity remains to be determined. Until infectivity tests are possible, saliva, urine, and stool from antigenemic persons should be considered infectious.

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