Salivary HBsAg in hepatitis B infection

BJØRN HURLEN, JON JONSEN, ALF NETLAND & SVEIN OSNES

Department of Microbiology, Dental Faculty, University of Oslo, and Department of Infectious Diseases, Oslo City Hospital

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Hepatitis B surface antigen (HBsAg) was detected by solid phase radioimmunoassay (RIA) in mixed saliva of 15 out of 50 antigenemic patients. The salivary antigen was present in low titers for a short period of time in the acute stage of illness. Occult blood was detected in most mixed saliva samples. In parotid saliva neither HBsAg nor occult blood was found. Salivary HBsAg is probably due to admixture of blood or exudate.

Key-words: Virology; immunology; saliva; blood

Bjørn Hurlen, Department of Microbiology, Dental Faculty, University of Oslo, P.O.B. 1052, Blindern, Oslo 3

Parenteral inoculation is still considered the most important mode of transmission of hepatitis B virus (HBV), and the most important source of HBV is blood from HBV infected patients and healthy carriers. However, the non-parenteral way of transmission has also been indicated (6, 13), and hepatitis B surface antigen (HBsAg) has been detected in body fluids other than blood (25). Thus a number of recent reports have shown that HBsAg may be present in saliva in seropositive individuals (3, 4, 7, 11, 12, 14, 17, 24). This is thought to be of epidemiologic importance particularly in institutions for the mentally retarded, among household contacts of HBV infected persons (21, 23) and possibly in dentistry (3).

The reported frequencies of HBsAg in saliva are rather inconsistent, ranging from 3.3 % (22) to 88 % (17). This may partly be due to variations in the methods used for collection, processing and testing the specimens for the presence of antigen. In some studies the saliva secretion was stimulated by parenteral administration of pilocarpine. In others the flow was accelerated by sucking inert materials like buttons or glass beads, by chewing paraffin pellets or by sour lemon candy or juice. Still others did not use artificial flow stimulation, and some studies were based on mouth washings or nasopharyngeal washings. Also, before testing for HBsAg the specimens had been treated by various means (ultracentrifugation, dialysis or lyophilization) or the test was performed on the untreated fluids.

In most studies HBsAg has been detec-

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ted by solid phase radioimmunoassay: immunodiffusion, counter immunoelectrophoresis and complement fixation tests have also been used.

In spite of the disagreement as regards the frequency of salivary HBsAg occurrence, there has been a general agreement towards lower HBsAg titers in saliva than in serum. Two reports (12, 16) have indicated that the peak concentrations of salivary HBsAg in cases of acute hepatitis B infection occur 1–2 months later than in serum. This has not been confirmed by others (21, 23) who have found it restricted to the acute stage of clinical illness.

A majority of the investigators studying this phenomenon have considered the possibility that blood contamination of saliva might be responsible for the occurrence of HBsAg. The specimens have therefore been tested for the presence of occult blood, most frequently by «dip and read» test strips based on an o-tolidine reaction, originally developed for detection of blood in urine. The occurrence of blood in mixed saliva has been frequent in some studies, infrequent in others.

The reports referred to concerned mixed saliva except for the report by Brodersen et al. (4) who also studied parotid saliva and found HBsAg in 16 out of 35 samples, obtained by introducing a probe into the parotid duct. Lemon juice was used as a gustatoric stimulation. The occurrence of occult blood in parotid saliva was not mentioned.

The aim of the present study was to evaluate the frequency, the duration and the relative concentration of HBsAg in mixed saliva and parotid fluid in hepatitis B infected patients using a radioimmunoassay.

MATERIALS AND METHODS

Fifty patients consecutively admitted to the Department of infectious diseases, Oslo City Hospital, with a diagnosis of hepatitis B were studied. The serologic diagnosis had been performed by The Microbiological Laboratory by a special RIA technique (5). They were 35 men and 15 women ranging in age from 16 to 73, mean age 29 years. However, more than 50 % of them were younger than 25 (Table 1).

Fourteen patients, 8 men and 6 women, suffering from non-B hepatitis served as a control. Their mean age was 23 years, ranging from 16 to 32 years.

Specimens of mixed saliva, parotid saliva and serum were collected weekly during the patients' stay in the hospital. The patients were asked to expectorate into widemouthed, ice-cooled plastic containers. On two occasions the patients were given paraffin pellets to accelerate the secretion.

Parotid saliva samples were obtained by means of suction cups (15) which allow unmixed saliva to flow from the sealed duct orifice through a catheter into test tubes. The tubes were kept on ice. All the saliva samples were collected within 1 $^1/_2$ hours preceding lunch. Blood samples were obtained by venepuncture in the morning the same day.

The samples were capped and kept on ice for 2–3 hours, tested for occult blood by Hemastix® (Ames), and stored at -40°C until tested for HBsAg. The sensitivity of the Hemastix test used for the present purpose was controlled, and the strips detected blood diluted in saliva at 1:512000. After thawing the specimens were tested for HBsAg by solid phase radioimmunoassay (Ausria II, Abbott, or Hepria-B, RIA International Inc) without further processing or concentration.

Parotid saliva samples were also concentrated by freeze-drying and reconstituted in distilled water to a concentration tenfold that of the original sample.

Table 1. *Age and sex distribution of patients examined for salivary hepatitis B surface antigen*

Age	HBsAg seropositive patients			HBsAg seronegative patients (controls)		
	M	F	Total	M	F	Total
16–25	19	9	28	5	6	11
26–35	6	4	10	2	0	2
36–45	3	0	3	0	0	0
46–55	5	0	5	0	0	0
55 +	2	1	3	0	0	0
Unknown	0	1	1	1	0	1
Total	35	15	50	8	6	14

RESULTS

From 50 patients seropositive for HBsAg 95 samples of mixed saliva were collected (Table 2). In 15 patients (30%) at least 1 saliva sample was found positive for HBsAg by radioimmunoassay. The positive samples were found in 10 men and 5 women with an age distribution consistent with the total study population.

In one patient salivary HBsAg was first detected in the fourth week of hospitalization. This patient appeared to develop a chronic hepatitis. In two patients the antigen occurred in saliva in the second week, otherwise salivary HBsAg was only seen in the first or in the first and second week.

The concentration of the antigen in mixed saliva was generally low compared to the concentration in serum. The counts per minute (cpm) in the gamma counter seldom exceeded 3 times the cutoff value of the test, i.e. the lowest value to be considered positive for HBsAg. In two-fold dilutions positivity seldom exceeded 2–3 dilution steps. For comparison the undiluted serum samples had peak values between 10 and 30 times the cutoff value, and by titration the difference was even greater. In 7 samples of saliva the cpm were slightly above the cutoff value, but were not positive at repeated tests. All positive samples were tested twice, and the specificity was confirmed by a blocking test.

Table 2. *Occurrence of HBsAg in mixed saliva samples from seropositive patients. Distribution by presence or absence of occult blood in the sample*

Occult blood	HBsAg positive	HBsAg negative	Total
Positive	20	70	90
Negative	1	4	5
Total	21	74	95

In 20 out of 21 HBsAg positive mixed saliva samples traces of blood were detected by Hemastix® test strips. However, occult blood was also detected in 70 out of 74 HBsAg negative samples of mixed saliva from seropositive patients.

Fiftysix specimens of parotid saliva obtained from 35 of the patients were all HBsAg negative when tested unconcentrated by RIA. Ten parotid saliva samples from patients with antigen in mixed saliva were concentrated by lyophilization, but remained negative. Occult blood was not detected in any of the parotid saliva samples.

Villarejos et al. (21) observed that the reactivity of saliva, expressed in cpm, progressively decreased with storage of the samples in refrigeration at 4°C. In the present study this was considered, and several samples were tested directly at the bedside of the patients but also after storage in the cold for about 3 hours. However, no significant differences were seen.

In the control group 14 samples of mixed saliva and 8 samples of parotid saliva were collected. All samples were HBsAg negative. Occult blood was detected in all samples of mixed saliva but in none of the parotid saliva samples.

DISCUSSION

There is reason to believe that solid phase radioimmunoassay methods are adequate for testing saliva specimens for HBsAg, with a sensitivity comparable to serum tests. Negative samples of serum and saliva showed the same cpm range and mean values. When equal amounts of positive control serum were added to negative serum and saliva samples and tested, no differences in cpm were seen.

In the present study salivary HBsAg occured in 30% of the seropositive patients. This frequency was lower than has previously been reported (3, 4, 7, 11, 17, 19, 23, 24). The disagreement is probably due to different processing techniques, which may bring subdetectable concentrations of HBsAg within detectable borders.

The occurrence of antigen was mainly limited to the acute stage of clinical illness, and it was found in low titers compared to serum. The presence of occult blood in nearly all the samples of mixed saliva indicates that the salivary HBsAg might originate from capillary leakage into the oral cavity. Spontanous bleeding and exudation is likely to occur in patients with poor oral hygiene and gingivitis, which is often seen in drug addicts. A number of the individuals investigated belonged to this group.

Thus saliva may be regarded as a diluent for blood or plasma and the degree of dilution determines whether HBsAg will occur in concentrations detectable by RIA or not. Occult blood is detected in dilutions beyond the border of RIA sensitivity to HBsAg. This probably explains the many samples of mixed saliva which were negative for antigen in spite of the presence of occult blood.

In the parotid samples neither HBsAg nor blood was detected. This supports the theory of salivary HBsAg being due to an admixture of blood and not secreted by the salivary glands at least not from the parotid gland.

From an epidemiological point of view the salivary antigen should be of the greatest concern in environments where susceptible individuals are frequently exposed to saliva, for instance in institutions for the mentally retarded because of their frequent drooling, in houshold contacts because of the frequency of both direct and indirect transmission of saliva, and in dentistry in which exposure to saliva is inevitable. However, the frequency of transmission to dentists is not invariably great (1,8) and seems to be related particularly to oral surgical procedures (9). The practical importance of salivary antigen depends on the frequency of occurrence, the duration and the concentration. In the present study low titers of salivary HBsAg was seen in 30% of the patients for a short period of time. Thus saliva seems to be of limited importance in the transmission of HBV.

REFERENCES

1. Aldershvile, J., Brock, A., Dietrichson, O., Hardt, F., Juhl, E., Madsbad, S., Mathiesen, L., Matzen, P., Nielsen, J.O., Schlichting, P., Sørensen, S. & Tage-Jensen, U. Hepatitis B virus infections among Danish dentists. J. Infec. Dis. 1978, 137, 63 – 66
2. Barker, L.F., Shulman, N.R. Murray, R., Hirschman, R.J., Ratner, F., Diefenbach, C.L. & Geller, H.M. Transmission of serum hepatitis. J. Am. Med. Assoc. 1970, 211, 1509 – 1512
3. Brodersen, M., Stegmann, S., Klein, K.-H., Trülzsch, D. & Rensch, P. Salivary HBsAg detected by radioimmunoassay. Lancet 1, 1974, 675 – 676
4. Brodersen, M., Wirth, M., Klein, K.-H. & Stegmann, S. Radioimmunologische Untersuchungen über die HBsAg-Ausscheidung in Speichel und Schweiss. Zentralbl. Bakt. Orig. A. 1976, 235, 316 – 319
5. Figenschau, K.J. & Ulstrup, J.C. Staphylococcal radioimmuno-assay for hepatitis B antigen and antibody. Acta Pathol. Microbiol. Scand. Sect. B. 1974, 82, 422 – 428
6. Giles, J.P., McCollum, R.W., Berndtson, L.V. Jr. & Krugman, S. Viral hepatitis. N. Engl. J. Med. 1969, 281, 119 – 122
7. Heathcote, J., Cameron, C.H. & Dane, D.S. Hepatitis B antigen in saliva and semen. Lancet 1, 1974, 71 – 73
8. Hurlen, B., Iversen, S.B. & Jonsen, J. Frequency of hepatitis in dental health personnel in Norway. Acta Odontol. Scand. 1979, 37, 189 – 193
9. Hurlen, B. & Iversen, S.B. Viral hepatitis in oral surgery and periodontics in Norway. Int. J. Oral. Surg. In press
10. Johnson, A.J., Semar, M., Newman, J., Harris, R.B., Brandt, D., Middleton, S. & Smith, J. Removal of hepatitis B antigen (HBsAg) from plasma fractions. J. Lab. Clin. Med. 1976, 88, 91 – 101
11. Kistler, G.S. Sonnabend, W. & Krech, U. Hepatitis B antigen (HB-Ag, Australia antigen) in mixed saliva of patients with HB antigenemia. Path. Microbiol. 1973, 39, 313 – 318
12. Kley, R., Kley, S., Bahre, G. & Lamberts, B. Ausscheidung von Hepatitis-B-Antigen im Speichel und Urine während des Verlaufes der akuten Virus-Hepatitis Typ B. Dtsch. Gesellsch. Inn. Med. 1975, 81, 1356 – 1358
13. Krugman, S., Giles, J.P. & Hammond, J. Infectious hepatitis. J. Am Med. Assoc. 1967, 200, 365 – 373
14. Marcolongo, R., Debolini, A & Barberi, A. HB antigen in saliva. Lancet 2, 1973, 1269
15. Mason, D.K. & Chisholm, D.M. Salivary glands in health and disease. W.B. Saunders Company Ltd., London, Philadelphia, Toronto, 1975, pp. 249 – 254
16. Ogra, P.L. Immunologic aspects of hepatitis-associated antigen and antibody in human body fluids. J. Immunol. 1973, 110, 1197 – 1205
17. Petersen, N.J., Barret, D.H., Bond, W.W., Berquist, K.R., Favero, M.S., Bender, T.R. & Maynard, J.E. Hepatitis B surface antigen in saliva, impetigous lesions and environment in remote Alaskan villages. Appl. Environ. Microbiol. 1976, 32, 572 – 574
18. Shirachi, R., Shiraishi, H., Matsumoto, S., Matsuda, S., Konno, T. & Ishida, N. Hepatitis B antigen in saliva. Tohoku J. exp. Med. 1973, 109, 201 – 202
19. Tanno, H., Fay, O.H., Roncoroni, M., Garcia, C. & Katz, R. Detection of Australia antigen in urine and saliva. Acta Hepatogastroenterol. 1974, 657, 373 – 376
20. Tiku, M.L., Beutner, K.R., Ramirez, R.I., Dienstag, J.L., Sultz, H.A. & Ogra, P.L., Distribution and characateristics of hepatitis B surface antigen in body fluids of institutionalized children and adults. J. Infect. Dis. 1976, 134, 342 – 347
21. Villarejos, V.M., Visoná, K.A., Gutiérrez, A. & Rodrïguez, A. Role of saliva, urine and feces in the transmission of type B hepatitis. N. Engl. J. Med. 1974, 291, 1375 – 1378
22. Vittal, S.B., Dourdourekas, D. & Steigmann, F. Hepatitis-B antigen in saliva, urine and tears. Am. J. Gastroenterol. 1974, 61, 133 – 135
23. Ward, R. Borchert, P., Wright, A. & Kline, E. Hepatitis B antigen in saliva and mouth washings. Lancet 2, 1972, 820 – 821
24. Wong, M.L., Lehmann, N.I. & Gust, I.D. Detection of hepatitis B surface antigen in the saliva of patients with acute hepatitis B, and of chronic carriers. Med. J. Aust. 1976, 2, 52 – 55
25. Zuckerman, A.J. Human viral hepatitis. Hepatitis-associated antigen and viruses. 2nd. ed. North Holland and American Elsevier, Amsterdam, Oxford, New York, 1975, pp 224 – 232