CLINICAL RESEARCH

Hepatitis B virus DNA in saliva, urine, and seminal fluid of carriers of hepatitis B e antigen

P KARAYIANNIS, D M NOVICK, A S F LOK, M J F FOWLER, J MONJARDINO, H C THOMAS

Abstract

Concentrated samples of saliva, urine, and seminal fluid from 23 men with chronic liver disease who were positive for hepatitis B e antigen were examined for the presence of hepatitis B virus deoxyribonucleic acid (HBV-DNA) by molecular hybridisation. HBV-DNA was detected in saliva from 15 of 17 men (88%), urine from 12 of 22 men (55%), and seminal fluid from 13 of 21 men (62%). The presence of hepatitis B virus in such secretions has important epidemiological implications for heterosexual and homosexual contact.

Introduction

The parenteral mode of transmission of hepatitis B virus is well established, but non-parenteral (or inapparent parenteral) routes of exposure may also play a part in infectivity; this may explain the high incidence of transmission of hepatitis B virus among homosexual men and family contacts of carriers of hepatitis B virus.¹⁻⁷ Several groups have therefore examined bodily fluids and secretions for the presence of hepatitis B surface antigen (HBsAg), which has been found in saliva,⁸⁻¹⁰ urine,⁹⁻¹² semen,⁸ breast milk,^{13 14} vaginal secretions and menstrual blood,^{15 16} and pancreatic and biliary secretions.¹⁷ There are conflicting reports of its presence in faeces.^{10 18-20} The detection of HBsAg alone, however, does not by itself denote potential infectivity as HBsAg may be found in the absence of infectious virions. The potential for transmission of

hepatitis B virus by inapparent parenteral means has therefore not been fully evaluated.

The presence of hepatitis B virus particles can be detected by molecular hybridisation techniques, which detect hepatitis B virus deoxyribonucleic acid (HBV-DNA).²¹⁻²⁵ The presence of HBV-DNA indicates potential infectivity. In this study we examined concentrated saliva, urine, and seminal fluid for the presence of HBV-DNA by molecular hybridisation. The results were correlated with serum concentrations of HBV-DNA and with the possible contamination of saliva and urine with blood.

Patients and methods

Twenty three patients with chronic hepatitis B virus infection (positive for HBsAg, hepatitis B e antigen (HBeAg), and HBV-DNA) who attended this hospital for antiviral treatment participated in the study. Two carriers positive for HBsAg and anti-HBe and a healthy subject negative for HBsAg served as controls. Eighteen of the participants provided samples of all the secretions that were requested (group 1). The remaining eight patients (group 2) either failed to provide some of the samples or provided insufficient samples.

Patients were issued with receptacles for the specimens and were asked to collect about 3 ml saliva when they woke up in the morning and before brushing their teeth; 50 ml urine voided in the morning; and a sample of semen obtained preferably after abstinence from sexual activity for three days. A specimen of serum was obtained on the same day by venepuncture.

All specimens were processed within two to three hours after collection. Specimens of saliva and urine were tested for the presence of blood by Hemastix (Ames Division, Miles Laboratories, Slough) before concentration. Samples of semen were allowed to liquefy at room temperature, and the sperm were then separated from the seminal fluid by centrifugation.

The saliva, urine, and seminal fluid were next placed in dialysis tubing and concentrated against macrogol 6000 (polyethylene glycol 6000) (BDH Chemicals, Poole). Saliva and seminal fluid were concentrated to 250 μ l irrespective of starting volume. Urine was concentrated from a 25 ml starting volume to 250 μ l (100-fold concentration). All samples were then stored at -20°C .

The methods of HBV-DNA extraction, preparation of radio-labelled probes by nick translation, and molecular hybridisation have been described previously.^{23 26-28} Viral DNA from saliva, urine, and seminal fluid was extracted as for serum. Autoradiographs

Academic Departments of Medicine and Physiology, Royal Free Hospital School of Medicine, London NW3 2PF

P KARAYIANNIS, BSC, PHD, research assistant

D M NOVICK, MD, research fellow

A S F LOK, MB, MRCP, research fellow

M J F FOWLER, BSC, PHD, research assistant

J MONJARDINO, MB, PHD, reader in physiology

H C THOMAS, PHD, FRCP, professor of medicine

Correspondence to: Professor H C Thomas.

were obtained by exposure of hybridised DNA on nitrocellulose filters for 24-48 hours. Samples from four patients were also analysed by agarose gel electrophoresis and Southern blotting followed by hybridisation with HBV-DNA labelled with phosphorus-32.²⁶

Results

The table shows the incidence of HBV-DNA in saliva, urine, and seminal fluid. HBV-DNA was detected in 55-88% of the samples from the HBeAg positive patients and none from the controls. Moreover, in group 1 at least one sample from every patient was positive for HBV-DNA and all 15 patients were therefore potentially infectious. Nine of these patients provided samples on a second occasion and similar positivity rates were obtained.

To confirm that the results obtained were due to HBV-DNA, samples were examined by Southern blotting to investigate the molecular species of the hybridisable DNA. Hybridisation signals in the form of smears covering the size range 2.0-2.8 kb were seen. These corresponded to the heterogeneous population of HBV-DNA associated with virions. The intensity of the autoradiography spots (figure) from all secretions was generally weak and required at least 48 hours of film exposure before becoming visible. This was particularly true for the samples of seminal fluid. Several specimens of saliva and urine, however, produced strong spots within 24 hours. Serum concentrations of HBV-DNA ranged from 8.94×10^{-11} to 2.77×10^{-9} mmol/l (186 to 5760 pg/ml).

Samples of urine and saliva were examined for blood. None of the samples of urine contained detectable amounts of blood. In the saliva, however, blood was detected in varying amounts (two trace, four small, two moderate, and three large) in 11 of the 17 samples positive for HBV-DNA.

Prevalence of HBV-DNA in saliva, urine, and seminal fluid. Figures are proportions of patients (and %)

	Saliva	Urine	Seminal fluid	Serum
Group 1	13/15 (86.6)	9/15 (60)	8/15 (53.3)	15/15 (100)
Group 2	2/2 (100)	3/7 (42.8)	5/6 (83)	8/8 (100)
Total	15/17 (88/2)	12/22 (54.5)	13/21 (61.9)	23/23 (100)
Controls	0/3	0/3	0/3	0/3

Composite autoradiograph of nitrocellulose filters bearing samples of saliva, urine, seminal fluid, and serum probed for HBV-DNA using HBV-DNA labelled with phosphorus-32. Black areas indicate presence of HBV-DNA in samples. Intensity of spot indicates amount of HBV-DNA present.

Discussion

The detection of HBV-DNA in saliva, urine, and seminal fluid by molecular hybridisation indicates the likely presence of hepatitis B virus particles in these secretions and establishes them as potential vehicles of infectivity. Although it has been suspected that non-parenteral transmission of hepatitis B virus may be due to contamination of such secretions with virions, this can now be substantiated by the sensitive technique of molecular hybridisation.

Of the concentrated saliva specimens, 87% were positive for HBV-DNA, and in some large amounts were present. The detection of HBV-DNA in some of these may have been due to the presence of oral or gingival lesions as blood was detected in 64% of the samples and this was gross in 31%. In some

cases, however, HBV-DNA was detected in saliva with no detectable contamination with blood. Of the concentrated urine specimens, 60% were positive for HBV-DNA and none were contaminated with blood. This prevalence was higher than previous reports of HBsAg in urine,^{9,10} possibly because of the greater degree of concentration achieved here and the more sensitive technique of molecular hybridisation. Seminal fluid was also found to be positive for HBV-DNA. The degree of concentration varied from twofold to 10-fold depending on the volume of the ejaculate.

The presence of HBV-DNA in the above secretions was independent of the amount of HBV-DNA in serum. Although no quantitative measurements were undertaken owing to the difficulty in standardising concentration factors, the amounts detected in the various secretions were small compared with those found in serum. Probably there is transudation or exudation of fluid containing virus from the general circulation into various body fluids rather than active virus replication at the site of secretion.

The presence of HBV-DNA in the various secretions was further confirmed by Southern blotting. Positive autoradiographs were obtained from smears of HBV-DNA sequences in the 2.0-2.8 kb region of the gels, where HBV-DNA associated with virions is normally found.²⁶

Most of the men investigated were homosexual, and all of them had at least one sample that was positive for HBV-DNA. It has been postulated that the high rate of transmission of hepatitis B virus in homosexual populations is due to contamination of partners with blood from lacerations in the rectum of the passive partner or cuts on the penis of the active partner.²⁰ Our results prove that this may not be the only mechanism. Contaminated semen or saliva deposited in the rectum during orogenital and oroanal contact might be alternative modes of transmission among homosexual men. Similarly, consorts of heterosexual men are at risk of infection from their partner's secretions. Indeed the potential of saliva and semen to transmit hepatitis B virus infection has been shown experimentally in primates.^{29,30}

References

- Dienstag JL. Toward the control of hepatitis B. *N Engl J Med* 1980;303:874-6.
- Szmunn W, Much MI, Prince AM, et al. The role of sexual behaviour in the spread of hepatitis B infection. *Ann Intern Med* 1975;83:489-95.
- Szmunn W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980;303:833-41.
- Redeker AG, Mosley JW, Gocke DJ, McKee AP, Pollack W. Hepatitis B immune globulin as a prophylactic measure for spouses exposed to acute type B hepatitis. *N Engl J Med* 1975;273:1055-9.
- Koff RS, Slavin MM, Connelly LJD, Rosen DR. Contagiousness of acute hepatitis B secondary attack rates in household contacts. *Gastroenterology* 1977;72:297-300.
- Inaba N, Ohkawa R, Matsuura A, Kudoh J, Takumizawa H. Sexual transmission of hepatitis B surface antigen. Infection of husbands by HBsAg carrier state wives. *Br J Vener Dis* 1979;55:366-8.
- Perillo RP, Belb L, Campbell C, et al. Hepatitis B e antigen, DNA polymerase activity, and infection of household contacts with hepatitis B virus. *Gastroenterology* 1979;76:1319-25.
- Heathcote J, Cameron CH, Dane DS. Hepatitis B antigen in saliva and semen. *Lancet* 1974;ii:71-5.
- Sung JL, Chen DS. Hepatitis B surface antigen in saliva, urine and ascites [Abstract]. *Hepatogastroenterol* 1983;30:183.
- Villarejos VM, Visona KA, Gutierrez A, Rodriguez A. Role of saliva, urine and feces in the transmission of type B hepatitis. *N Engl J Med* 1974;291:1375-8.
- Blainey JD, Earle A, Flewett TH, Williams LKL. Is the urine infective in serum hepatitis? *Lancet* 1971;ii:797.
- Tripathi I, Horst HG. Detection of Australia-SH-antigen in urine. *Nature* 1971;231:266-7.
- Linnemann CC, Goldberg S. HBsAg in breast milk. *Lancet* 1974;ii:155.
- Boxall EH, Flewett TH, Dane DS, Cameron CH, MacCallum FO, Lee TW. Hepatitis B surface antigen in breast milk. *Lancet* 1974;ii:1007-8.
- Darani M, Gerber M. Hepatitis-B antigen in vaginal secretions. *Lancet* 1974;ii:1008.
- Mazzur S. Menstrual blood as a vehicle of Australia antigen transmission. *Lancet* 1973;ii:749.
- Hoefs JC, Renner IG, Ashcavi M, Redeker AC. Hepatitis B surface antigen in pancreatic and biliary secretions. *Gastroenterology* 1980;79:191-4.
- Feinman SV, Berris B, Rebane A, Sinclair JC, Wilson S, Wrobel D. Failure to detect hepatitis B surface antigen in faeces of HBsAg-positive persons. *J Infect Dis* 1979;140:407-10.
- Moodie JW, Stannard LM, Kipps A. The problem of the demonstration of hepatitis B antigen in faeces and bile. *J Clin Pathol* 1974;27:693-7.
- Reiner NE, Judson FN, Bond WW, Francis DP, Petersen NJ. Asymptomatic rectal mucosal lesions and hepatitis B surface antigen at sites of sexual contact in homosexual men with persistent hepatitis B virus infection. *Ann Intern Med* 1984;96:170-3.
- Brechot C, Scotto J, Charnay P, et al. Detection of hepatitis B virus DNA in liver and serum. A direct appraisal of the carrier state. *Lancet* 1981;ii:765-7.
- Bonino F, Hoyer B, Nelson J, Engle R, Verme G, Gerin J. Hepatitis B virus

- DNA in the sera of HBsAg carriers: a marker of active hepatitis B virus replication in the liver. *Hepatology* 1981;1:386-91.
- 23 Weller IVD, Fowler MJF, Monjardino J, Thomas HC. The detection of HBV-DNA in serum by molecular hybridisation: a more sensitive method for the detection of complete HBV particles. *J Med Virol* 1982;9:273-80.
- 24 Scotto J, Hadchouel M, Hery C, Yvart J, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in serum by a simple spot hybridisation technique. Comparison with results for other viral markers. *Hepatology* 1983;3:279-84.
- 25 Lieberman HM, La Brecque DR, Kew MC, Hadziyannis SJ, Shafritz DA. Detection of hepatitis B virus DNA directly in human serum by a simplified molecular hybridisation test: comparison to HBeAg/anti-HBe status in HBsAg carriers. *Hepatology* 1983;3:285-91.
- 26 Monjardino J, Fowler MJF, Montano L, *et al.* Analysis of hepatitis virus DNA in the liver and serum of HBe antigen positive chimpanzee carriers. *J Med Virol* 1982;9:189-99.

- 27 Karayiannis P, Fowler MJF, Lok ASF, Greenfield C, Monjardino J, Thomas HC. Detection of serum HBV-DNA by molecular hybridisation: correlation with HBeAg/anti-HBe status, racial origin, liver histology and hepatocellular carcinoma. *J Hepatol* 1985;1:99-106.
- 28 Weinstock R, Sweet R, Weiss M, Cedar R, Axel R. Intragenic DNA spacers interrupt the oralbumin gene. *Proc Natl Acad Sci USA* 1978;75:1295-303.
- 29 Bancroft WH, Snitbhan R, Scott RM, *et al.* Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. *J Infect Dis* 1977;135:79-85.
- 30 Alter HJ, Purcell RH, Gerin JL, *et al.* Transmission of hepatitis B to chimpanzees by hepatitis B surface antigen-positive saliva and semen. *Infect Immun* 1977;16:928-33.

(Accepted 28 February 1985)

Chronic ulceration of the leg: extent of the problem and provision of care

M J CALLAM, C V RUCKLEY, D R HARPER, J J DALE

Abstract

A postal survey in two health board areas in Scotland, encompassing a population of about one million, identified 1477 patients with chronic ulcers of the leg. Women outnumbered men by a ratio of 2.8:1. The median age of the women was 74 and of the men 67. Seventy two (5%) were hospital inpatients, 174 (12%) were managed jointly by the primary care team and outpatient departments, and 1201 (83%) were managed entirely in the community.

Efforts to improve the management of chronic ulcers of the leg should focus on primary health care.

Introduction

Chronic ulceration of the leg appears to have perplexed physicians since medical records began. Although there are many reports on its management, little information is available on the overall size and extent of the problem or its clinical course.

Two European surveys into venous disease have been made, providing data on the prevalence of leg ulceration. The first was a study, based on a questionnaire, of the adult population of Klatov in Bohemia in 1961 by Bobek *et al.*¹ Those people who indicated that they had evidence of venous disease were subsequently examined. This survey showed that the prevalence of leg ulceration, either open or healed, was 1%. The second and more recent study, of factory workers in Basle, Switzerland, showed a similar proportion.²

Information concerning the United Kingdom is almost non-existent. In 1929 Dickson-Wright suggested a prevalence of 0.5% but admitted that this figure was an estimate.³ In 1951 Boyd *et al.* arrived at a similar figure based on the returns of patients registered as off work due to leg ulceration,⁴ but this figure was probably an underestimate. In a study supplementary to that

reported here and based on patients in a group practice in Edinburgh we recently estimated that 1% of adults suffer from chronic leg ulcers.⁵

Much of the information in reports on leg ulcers is based on small, selected populations, usually patients attending outpatient departments. An understandable conclusion that might be drawn from such reports is that all leg ulcers are managed by dermatologists, surgeons, or physiotherapists. A preliminary survey by our own group, however, showed that most patients were cared for in the community by district nurses and represented a formidable problem for the primary care services not only in terms of numbers but also in various aspects of management. We concluded that the first step towards improving care was to obtain better information on the scale of the problem, the clinical course of the condition, and how care was being provided.

The Lothian and Forth Valley leg ulcer study was therefore set up in 1981. This paper reports the first phase, the main aims of which were to establish a point prevalence of leg ulcers and to find out who was providing the care.

Methods

The survey was carried out in 1981-2 in the neighbouring health board areas of Lothian and Forth Valley, which have a mixed urban and rural population of about one million. The objective was to identify all patients receiving treatment for chronic leg ulceration from any branch of the National Health Service at the time of the survey.

To identify all patients receiving treatment in the community recording forms were sent to all general practitioners, district and occupational nurses, and wardens of old people's homes. To identify patients receiving either outpatient or inpatient care at a hospital forms were sent to outpatient departments, physiotherapy departments, and inpatient wards of general hospitals and all acute and long stay hospitals. In each case the correspondent was asked to identify all the patients currently undergoing treatment for active chronic leg ulceration or who had received treatment within three months. Efforts were made to obtain replies from non-responders by means of follow up letters and telephone calls.

The resulting returns were cross checked to ensure that patients reported from more than one source were included only once in the final total.

Results

Of the 572 general practitioners approached, only 37 refused to cooperate with the study. Complete returns were obtained from all other correspondent groups. Notifications of 2128 patients were received, of which 651 were reported from more than one source, giving a total of 1477 patients (1765 (83%) were reported by general practitioners, district nurses, occupational health services, and old people's homes; and 104 (5%) by physiotherapy departments, 148 (7%) by

Leith Hospital, Edinburgh

M J CALLAM, MB, FRCS, senior surgical registrar

Vascular Surgical Unit, Royal Infirmary, Edinburgh

C V RUCKLEY, MB, FRCS, consultant surgeon

Surgical Unit, Royal Infirmary, Falkirk

D R HARPER, MD, FRCS, consultant surgeon

Department of Nursing, Lothian Health Board, Edinburgh

J J DALE, SRN, MSC, senior nursing officer

Correspondence to: Mrs J J Dale, Lothian and Forth Valley Leg Ulcer Study, 40 Colinton Road, Edinburgh.