RESEARCH NOTE

DETECTION OF HbsAg AND HBV DNA IN SERUM AND SALIVA OF HBV CARRIERS

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Abstract. Serum and saliva samples from 23 patients known to be HBsAg-positive HBV carriers and 17 healthy control subjects were analyzed for hepatitis B virus (HBV) by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). All serum samples of the HBV carriers were positive for HBsAg, with 21 also positive for HBV DNA. In comparison, 22 saliva samples of HBV carriers were positive for HBsAg whereas only 11 of the 23 tested were positive for HBV DNA. Based on these results we have arrived at the conclusion that the saliva of HBV carriers might be potentially infectious and also that saliva testing could serve as an alternative technique for identifying HBV carriers.

On a global scale, chronic hepatitis B virus infection represents the major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Therefore, particularly in developing countries with the highest prevalence of chronic HBV infection, the virus incurs a high incidence of morbidity and mortality. For HBV carriage to become established infection has to occur either at birth or during early infancy. In frequency exceeding horizontal transmission among family members, pre-school children or, more generally, young children more or less permanently exposed to close contact with their peers of a similar age, vertical transmission from chronically infected HBsAg-positive mothers to their newborns constitutes the major cause of ensuing chronic HBV carriage. Among infants born to HBsAg/HBeAg-positive carrier mothers chronic HBV infection can be prevented by administering hepatitis B vaccine (Poovorawan, 1989). Up to now, the serologic assay for HBsAg has been routinely applied in clinical practice to diagnose HBV infection, but a non-invasive method for testing pregnant women would be infinitely preferable, especially in developing countries where many patients are reluctant to provide blood samples. Based on the observation that HBV can be transmitted by parenteral exposure to human body fluids such as breast milk, semen and saliva (Lee *et al*, 1978; Scott *et al*, 1980), various groups have undertaken to design a detection assay for HBsAg in human saliva (Richards *et al*, 1996; Parry *et al*, 1987; Thieme *et al*, 1992). The test thus devised can provide a simplified alternative, at least as far as obtaining the samples, and more significantly, potentially infectious individuals can easily be identified, especially as HBV transmission in saliva has been shown to occur through breaks in the skin (Scott *et al*, 1980).

Whole mouth saliva and serum were obtained from 23 subjects previously identified as HBsAg -positive carriers, comprising 12 females and 11 males with their age ranging from 13 to 46 years, and 17 control subjects, comprising 14 females and 3 males with their age ranging from 10 to 50 years. The specimens thus obtained were stored at -20ºC until further analysis. All serum and saliva samples were tested for HBsAg applying a commercially available kit (Auszyme Monoclonal enzyme immunoassay (EIA); Abbott Laboratories, North Chicago, Ill) according to the manufacturer's instructions. In addition, all samples were also examined for the presence of HBV DNA by nested polymerase chain reaction using 2 sets of primers, primers F1 and R6 in the first amplification round and primers F2 and R5 in the second, respectively. The primer sequences applied were as follows: F1 5′-GGAGCGGGAGCATTCGGGCCA-3′ (nt position 3022-3042), R6 5′-GGCGAGAAAGTGAAA

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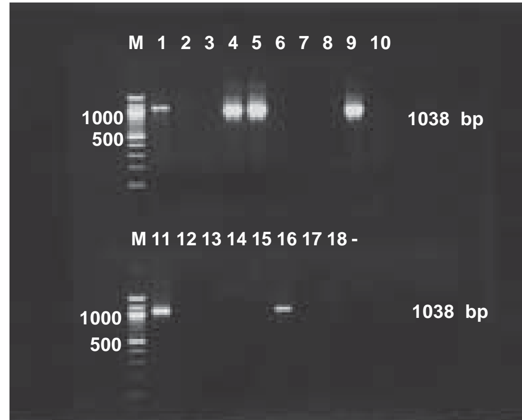
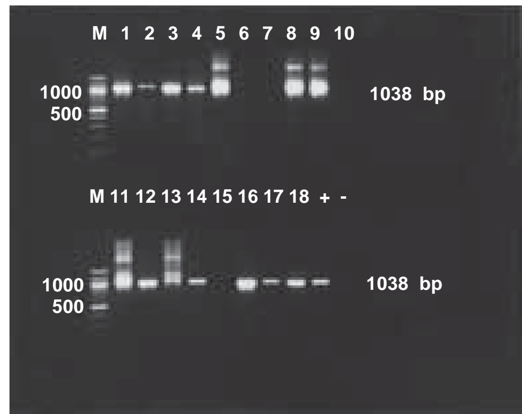
GCCTG-3′ (nt position 1103-1084), F2 5′-CATCC TCAGGCCATGCAGTGGA-3′ (nt position 3193-3214), R5 5′-AGCCCAAAAGACCCAGAATTC -3′ (nt position 1015-995). The HBV DNA was isolated and amplified as previously described (Theamboonlers *et al*, 1999). After electrophoresis on a 1.5% agarose gel with ethidium bromide added upon preparation the amplified samples were visualized under UV light.

All 23 serum samples from the HBsAg-positive subjects were positive for HBsAg and 21 of those were positive for HBV DNA whereas those from the control subjects were negative for both markers. The results of HBsAg and HBV DNA obtained from saliva specimens are shown in Table 1. The size of the PCR products obtained from both serum and saliva specimens was 1038 bp as shown in Fig 1.

Hepatitis B virus (HBV) has been well known for its parenteral and sexual dissemination. Applying ELISA or PCR it has also been detected in saliva (Richards *et al*, 1996; Parry *et al*, 1987; Ljunggren *et al*, 1993.) Whether HBV can be transmitted orally is still a matter of debate. The high prevalence of HBV among dentist personnel, children in institutions and family members suggests that HBV can be spread by saliva (Mosley *et al*, 1975; Szmuness and Prince, 1971; Szmuness *et al*, 1973; Mitch *et al*, 1974; Heathcote *et al*, 1974). This has been confirmed by reports on HBV transmission by a human bite (Mac Quarrie *et al*, 1974; Stornello, 1991). In experimental studies using gibbons, HBV transmission occurred when HBV-positive human saliva was administered parenterally, but not orally, irrespective of the trauma of tooth-brushing even upon application of large amounts of saliva (Scott *et al*, 1980; Bancroft *et al*, 1977).





M = Molecular weight marker; +/- = Positive/negative control
No. 1-5, 8-9, 11-14, 16-18 = HBsAg-positive subjects.
No. 6, 7, 10, 15 = Control subjects.

Fig 1–Polymerase chain reaction (PCR) of HBV DNA from saliva (above) and serum (bottom) of HBsAg-positive and control subjects.

Table 1
Analysis of saliva from patient (HBsAg - positive) and control subjects by ELISA and PCR of the HBV DNA in serum and saliva.

	Serum				Saliva			
	HBsAg		HBV DNA		HBsAg		HBV DNA	
	+	-	+	-	+	-	+	-
Patient n=23 HBsAg-positive	23	0	21	2	22	1	11	12
Control n=17 (HBsAg-negative)	0	17	0	17	0	17	0	17

This infrequent transmission might be due to low concentrations of infectious virus in saliva and/or to an at least partial reduction in its virulence by the primary immune response inherent in saliva.

We found the amount of HBsAg based on the sample/cut-off ratio to be higher in serum (mean $\pm$ SD $\geq$ 69.96 $\pm$ 14.29) than in saliva (mean $\pm$ SD = 37.61 $\pm$ 24.28). ELISA for HBV detection performed in saliva has several benefits as compared to serology. It is inexpensive, non-invasive, useful in epidemiological studies and convenient as it can be performed in the clinic or at the bedside. Moreover, with this simple test the potential infectivity of any given individual can easily be assessed. Based on the high sensitivity of ELISA in saliva, this method appears appropriate for screening. We could detect HBV DNA in saliva of 11 HBsAg - positive subjects whose saliva was ELISA positive. It is possible that HBV in saliva of those subjects were an incomplete virus containing only the surface antigen. In conclusion our observations have emphasized the risk of saliva from HBsAg-positive patients to be potentially infectious. Further investigations are required to confirm the infectivity of human saliva, as well as determine the characteristics of HBV present in saliva.

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