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NOTE

Detection of Hepatitis B Virus and *Mycobacterium tuberculosis* in Korean Dental Patients

Sun-A Lee[1,†], So Young Yoo[2,†], Kee-Sung Kay[1] and Joong-Ki Kook[2,*]

[1]*Department of Prosthodontic Dentistry and*
[2]*Department of Oral Biochemistry, College of Dentistry, Chosun University, Gwang-ju 501-759, Korea*

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This study examined the detection rate of the hepatitis B virus (HBV) and *Mycobacterium tuberculosis* (Mtb) in serum and saliva samples, respectively, from 120 dental patients who were unaware if they have or had either hepatitis or tuberculosis. The frequencies of HBsAg and anti-HBs were determined using an immunochromatic assay. Mtb positivity was determined by the PCR method. Of the 120 patients, 7 (5.8%) were HBV positive and 30 (25.0%) were Mtb positive. This highlights the fact that dental health care workers (DHCWs) can be exposed to the risk of infection from blood- or saliva-borne pathogens as a consequence of their work. Therefore, it is very important to prevent cross infection between patients and dental personnel. Accordingly, laboratory tests prior to surgical treatment are needed to determine the infectious state of dental patients in order to prevent the transmission of infectious diseases in dental clinics.

Key words: Dental patients, detection, hepatitis B virus, *Mycobacterium tuberculosis*

Dental health care workers (DHCWs) are at risk of infections from blood- or saliva-borne pathogens, including the hepatitis B virus (HBV) and *Mycobacterium tuberculosis* (Mtb) (Korean Academy of Family Medicine, 1995; Araujo and Andreana, 2002; Eguchi *et al.*, 2003). The HBV belongs to the genus *Hepadnavirus* in the family *Hepadnaviridae*. It is an enveloped virus with a particle diameter of 42 to 45 nm (Juszczyk, 2000; Shim *et al.*, 2001), and its genome consists of double-stranded, linear DNA (Hollinger, 1996). The HBV has been reported to be the most prevalent blood-borne pathogen (Beltrami *et al.*, 2000; Rosen, 2000; Bae *et al.*, 2002). This virus can induce a chronic carrier state, liver cirrhosis and even cancer (Bradley, 1999; Mitra, 1999). Viral hepatitis can be transmitted between patients, dentists, and dental hygienists. The horizontal spread of these viruses during dental procedures can occur as a result of contact with patients' blood or saliva in which prior contact to an infected individual had occurred, an injury from a contaminated needle or sharp instrument, and the inspiration of flying debris from the oral cavity. There are more than 350 million chronic HBV carriers worldwide (World Health Organization, 1997). In Korea, the percentage of HBV carriers has been reported to range from 3 to 6% (Shin and Kim, 1994; Korean Academy of Family Medicine, 1995). This rate is greater than those reported in many developed countries, which means that there is a higher probability of HBV transmission from a patient to a dentist in Korea.

Tuberculosis (TB) in humans is an infection caused mainly by two species of bacteria: Mtb and *M. bovis* (Samaranayake, 2002). Recently, there have been reports of mycobacterial infections other than tuberculosis (MOTT) including *M. avium* and *M. intracellulare* (*M. avium-intracellulare* complex (MAC), and *M. scrofulaceum* and *M. haemophilum* (Samaranayake, 2002). TB is spread via airborne droplets containing bacilli, typically as a result of coughing, sneezing, or even talking (Samaranayake, 2002). In dental clinics, DHCWs have a high risk of contracting Mtb infections owing to their close contact with patients and aerosol particles during the dental treatment process (Eguchi *et al.*, 2003).

Many patients who visit dental clinics are unaware if they are infected with infectious pathogens, such as HBV or Mtb. In addition, these patients are not always assessed for these infections, either before or after treatment. This is despite the fact that HBV or Mtb infected patients routinely visiting dental clinics. Consequently, DHCWs un-

[†]*Contributed equally*
* To whom correspondence should be addressed.
(Tel) 82-62-230-6877; (Fax) 82-62-224-3706
(E-mail) jkkook@chosun.ac.kr

knowingly treat these patients. Therefore, laboratory tests for several infectious diseases including viral hepatitis and tuberculosis need to be performed prior to dental treatment. Unfortunately, dental patients are seldom required to undergo blood sampling in dental clinics and dental hospitals. In addition, the National Health Insurance Corporation in Korea does not approve of blood analysis for patients with periodontitis, endodontic infections, and dental caries. In order to highlight the need for screening prior to a dental examination, this study examined the detection rate of the HBV and Mtb in the serum and saliva, respectively, from patients who were unaware if they have or had hepatitis or TB.

One hundred and twenty patients (58 males and 62 females; aged 17 to 78, mean 35.5) with either gingivitis or chronic periodontitis, who visited Dr. Lee Sun-A's dental clinic at Gwang-ju in Korea from January 2002 to October 2002, were enrolled in this study. All of the patients were unaware if they were infected with the HBV or Mtb. In order to detect the HBV, a 3 ml sample of venous bloods was drawn from the cubital fossa of the right arm from each of the 120 patients using sterile techniques. The blood samples were then centrifuged and the serum fractions were stored at -20°C until needed. In order to detect the tuberculosis pathogens, 1 ml of saliva was obtained from all of the patients. The saliva samples were also stored at -20°C until needed.

Tests for the HBV surface antigen (HBsAg) and the antibody to the HBV surface antigen (anti-HBs) were performed using an immunochromatographic assay (ICA) with an ASAN Easy Test *HBs Strip* (ASAN Pharmaceutical, Korea) and an ASAN Easy Test Anti-HBs Strip (ASAN Pharmaceutical, Korea), respectively, according to the manufacturer's instructions.

In order to detect mycobacterial DNA, the bacterial genomic DNA from each patient's saliva was prepared using a slight modification of the bead beater-phenol extraction method (Kim *et al.*, 1999). A 100 sample of saliva was mixed with 100 µl of 2×TEN buffer (200 mM Tris-HCl, 2 mM EDTA, 200 mM NaCl; pH 8.0), placed in a 2.0 ml screw-cap microcentrifuge tube filled with 100 µl (packed volume) of glass beads (diameter, 0.1 mm; Biospec Products, USA) and 100 µl phenol-chloroform isopropyl alcohol (50 : 49 : 1). The tube was oscillated on a Mini-Bead Beater (Biospec Products, Canada) for 1 min to disrupt the bacteria, and the tube was then centrifuged (12,000×g, 5 min) to separate the phases. After the aqueous phase had been transferred into another clean tube, 10 µl of a 3 M sodium acetate solution and 250 µl of ice-cold ethanol were added to the aqueous phase. After the tube was further centrifuged (12,000×g, 10 min, 4°C), the DNA pellet was washed with 70% ethanol, dissolved in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0), and used as a template for PCR.

Detection of Mtb (amplicon size; 155 bp) was performed by nested PCR according to the method described by Pierre *et al.* (1991). The PCR reaction was performed using an *AccuPower*®PCR PreMix (Bioneer, Korea), which contained 5 nmole of each deoxynucleoside triphosphate, 0.8 µmole KCl, 0.2 µmole Tris-HCl (pH 9.0), 0.03 µmole $MgCl_2$, and 1 unit of *Taq* DNA polymerase. In the first amplification, 4 µl of the purified DNA sample and 20 pmoles of each of the oligonucleotide primers TB-1 (5'-GAG ATC GAG CTG GAG GAT CC-3') and TB-2 (5'-AGC TGC AGC CCA AAA GGT GTT-3') were added to the PCR PreMix tube. PCR was performed in a final volume of 20 µl. The PCR reaction was run for 30 cycles on a Peltier thermal cycler (Model PTC-200 DNA engine™, MJ Research, USA) under the following conditions: denaturation at 95°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 1 min. The final cycle included an additional extension time of 10 min at 72°C. The second PCR reaction was carried out for 30 cycles with the primers 20 pmoles of each of the oligonucleotide TB-28 (5'-CCA TCG ATC CGA GAC CCT GCT CAA GGG C-3') and TB-29C (5'-TGC TCT AGA CTC CTC GAC GGT GAT GAC G-3'), and 1 µl of each of the original amplification product as described above. A 4 µl aliquot of the reaction mixture was analyzed by 2.0% agarose gel electrophoresis in Tris-acetate buffer (40 mM Tris-acetate, 1 mM EDTA, [pH 8.0]) at 100 V for 30 min. The amplification products were stained with ethidium bromide and visualized by UV transillumination.

Of the 120 patients, 7 (5.8%) tested positive for HBsAg and 53 (44.2%) were anti-HBs positive (Table 1, 2). These results showed that the percentage of HBV carriers in the 120 patients, who were unaware that they were infected with the HBV, was 5.8%. This value is consistent with previous reports from Korea (Korean Academy of Family Medicine, 1995; Samaranayake, 2002). PCR was also performed using a commercial detection kit (data not shown) in order to detect HBV DNA from both the saliva and serum samples. The data showed that many more non-specific bands were produced from the saliva samples than from the serum samples. The reason for this is unclear. However, it may have been due to the existence of many types of bacterial genomic DNA in the saliva reacting with the PCR primers. In the saliva samples, neither the HbsAg antigen nor the anti-HBs antibody was detected in any of the patients (data not shown). This discrepancy may have been due to a low sensitivity of ICA in detecting HBsAg and anti-HBs from the saliva samples or to the lower concentration of these molecules in the saliva than in the serum.

Mtb was detected in saliva samples from 30 out of 120 patients (25.0%) (Table 1). However, this study could not determine if these patients were in the active state. This high detection rate might be due to the fact that PCR can detect viable as well as dead cells. It has been reported that the detection rate of nested PCR for Mtb is 50% in

Table 1. Detection of viral hepatitis and tuberculosis pathogens in the serum or saliva of patients

No.	Sex	Age	HBsAg in serum	Anti-HBs in serum	Mtb* in saliva	No.	Sex	Age	HBsAg in serum	Anti-HBs in serum	Mtb* in saliva
1	F	45	+	−	−	61	M	24	−	−	−
2	M	32	−	−	+	62	M	70	−	−	+
3	M	35	−	−	−	63	F	32	−	−	+
4	F	38	−	+	−	64	M	60	−	+	−
5	M	34	−	−	+	65	M	37	−	+	+
6	M	30	−	−	−	66	F	33	−	−	−
7	M	26	−	−	−	67	M	18	−	−	−
8	M	26	−	+	−	68	M	39	−	−	+
9	F	50	−	+	−	69	F	19	−	+	+
10	F	54	−	+	−	70	M	26	−	+	+
11	F	54	−	−	−	71	F	23	−	−	−
12	M	47	−	−	−	72	M	26	−	−	+
13	F	51	−	−	−	73	M	37	−	+	+
14	F	47	−	−	−	74	M	23	−	−	+
15	F	52	−	−	−	75	F	35	−	+	+
16	F	58	−	+	−	76	M	28	−	−	−
17	M	54	−	−	−	77	M	26	−	−	+
18	F	23	−	−	−	78	F	19	−	+	−
19	M	26	−	+	−	79	F	26	−	−	−
20	M	25	−	−	−	80	F	20	−	−	−
21	M	58	−	−	+	81	F	33	−	+	−
22	M	27	+	−	−	82	F	39	−	+	+
23	F	20	−	+	−	83	F	21	−	+	+
24	M	30	−	+	−	84	F	30	−	+	−
25	M	53	−	+	−	85	M	55	−	−	−
26	M	44	+	−	−	86	F	23	−	+	+
27	M	57	−	+	−	87	M	25	−	−	−
28	M	17	−	−	−	88	F	25	−	+	−
29	M	78	−	−	−	89	M	41	−	−	−
30	F	20	−	−	−	90	M	29	−	+	−
31	M	42	−	+	−	91	M	30	−	+	−
32	M	41	−	+	−	92	M	42	−	−	−
33	M	64	−	+	−	93	F	46	−	+	−
34	M	24	−	−	+	94	M	27	−	−	−
35	F	22	−	+	−	95	F	54	−	+	−
36	F	54	−	+	−	96	F	25	−	+	−
37	F	65	−	−	+	97	F	27	−	−	−
38	M	36	−	−	−	98	F	43	−	−	−
39	F	20	−	+	+	99	F	39	−	+	−
40	F	44	−	−	+	100	M	33	−	−	−
41	M	37	+	−	+	101	M	25	−	−	−
42	F	54	−	+	+	102	F	20	−	+	−
43	F	34	−	−	−	103	M	40	−	−	−
44	F	28	−	−	+	104	M	49	−	−	−
45	M	49	−	+	−	105	M	24	−	+	−
46	M	34	−	+	+	106	F	43	−	−	−
47	F	22	+	−	+	107	M	26	−	+	−
48	M	18	−	−	+	108	M	25	−	+	+
49	M	41	−	−	−	109	F	27	−	−	−
50	M	18	−	−	−	110	F	22	−	+	−
51	F	42	−	−	−	111	M	21	−	−	−
52	F	37	−	−	−	112	M	24	−	+	+
53	M	76	−	+	−	113	M	36	−	−	+
54	M	37	−	−	−	114	F	34	−	−	−
55	F	23	−	+	−	115	F	23	−	+	−
56	F	24	−	−	−	116	M	20	+	−	−
57	M	37	−	+	−	117	F	20	−	+	−
58	F	38	−	−	−	118	F	24	−	+	−
59	F	53	+	−	−	119	F	31	−	+	−
60	F	64	−	+	−	120	F	26	−	+	−

**Mycobacterium tuberculosis*

Table 2. Summary of the detection rate of HBV and TB pathogens

	Hepatitis B Virus in serum			*Mycobacterium tuberculosis* in saliva
	HBsAg(−) Anti-HBs(−)	HBsAg(+) Anti-HBs(−)	HBsAg(−) Anti-HBs(+)	
No of samples (n=120)	60	7	53	30
%	50.0	5.8	44.2	25.0

mixed saliva samples after chemotherapy, but no Mtb was detected using the culture method (Eguchi *et al.*, 2003). Unfortunately, at the time of this study, there was no epidemiological data available highlighting the prevalence ratio of Mtb DNA in saliva among the general population in Korea. As a result, we could not present comparative data in this study.

Many patients refused to participate in the study because they were either frightened of the pain associated with blood sampling or they knew that they were infected with either the HBV or TB pathogens. Therefore, it is possible that the true detection rate of these infectious pathogens in dental patients could have been even higher.

Recently, it was reported that approximately 88.7% and 4.5% of dentists in Korea wear a mask and gloves during treatment, respectively (Song *et al.*, 1999). In contrast, the percentage of hygienists wearing a mask and gloves with all patients is 13.6% and 0.9%, respectively. This shows that basic barrier techniques for preventing cross-infections are not being used consistently. Accordingly, nationwide guidelines for barrier techniques need to be developed and hepatitis vaccinations should be given to dental personnel.

These results suggest that DHCWs can be infected with these blood- or saliva-borne pathogens during dental treatment processes such as flap operations, scaling using hand instruments or an ultrasonic scaler, tooth extractions, and many others. The results in this study are very important for assisting dentists in preventing the horizontal transmission from infected patients to dental personnel in dental clinics. In addition, laboratory tests prior to surgical treatment are needed to determine the possible infectious condition of dental patients.

References

Araujo, M.W. and S. Andreana. 2002. Risk and prevention of transmission of infectious diseases in dentistry. *Quintessence Int.* 33, 376-382.

Bae, C.S., G.Y. Lim, J.S. Kim, and B.K. Hur. 2002. Quadrivalent combined vaccine, including diphtheria toxoid, tetanus toxoid, detoxified whole cell pertussis, and hepatitis B surface antigen. *J. Microbiol. Biotechnol.* 12, 338-343.

Beltrami, E.M., I.T. Williams, C.N. Shapiro, and M.E. Chamberland. 2000. Risk and management of blood-borne infections in health care workers. *Clin. Microbiol. Rev.* 13, 385-407.

Bradley, D.W. 1999. Hepatitis viruses: Their role in human cancer. *Proc. Assoc. Am. Physicians* 111, 588-93.

Eguchi, J., K. Ishihara, A. Watanabe, Y. Fukumoto, and K. Okuda. 2003. PCR method is essential for detecting *Mycobacterium tuberculosis* in oral cavity samples. *Oral Microbiol. Immunol.* 18, 156-159.

Hollinger, F.B. 1996. Comprehensive control (or elimination) of hepatitis B virus transmission in the United States. *Gut* 38(Suppl 2), S24-S30.

Juszczyk, J. 2000. Clinical course and consequences of hepatitis B infection. *Vaccine* 18(Suppl. 1), S23-S25.

Kim, B.J., S.H. Lee, M.A. Lyu, S.J. Kim, G.H. Bai, G.T. Chae, E.C. Kim, C.Y. Cha, and Y.H. Kook. 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). *J. Clin. Microbiol.* 37, 1714-1720.

Koran Academy of Family Medicine. 1995. Disease prevention and health promotion in Korea: Hepatitis B, pp. 134-140, Korea Medical Publishing Co., Seoul.

Leontiou, A.P., M.M. Coogan, and S. Aspinall. 1999. Disinfection of dental diamond burs contaminated with hepatitis B virus. *J. Prosthet. Dent.* 82, 332-335.

Mitra, A.K. 1999. Hepatitis C-related hepatocellular carcinoma: Prevalence around the world, factors interacting, and role of genotypes. *Epidemiol. Rev.* 21, 180-187.

Pierre, C., D. Lecossier, Y. Boussougant, D. Bocart, V. Joly, P. Yeni, and A.J. Hance. 1991. Use of a reamplification protocol improves sensitivity of detection of *Mycobacterium tuberculosis* in clinical samples by amplification of DNA. *J. Clin. Microbiol.* 29, 712-717.

Rosen, H.R. 2000. Retransplantation for hepatitis C: implications of different policies. *Liver Transpl.* 6(Suppl. 2), S41-S46.

Samaranayake, P. 2002. Re-emergence of tuberculosis and its variants: implications for dentistry. *Int. Dent. J.* 52, 330-336.

Shim, J.K., D.W. Kim, T.H. Chung, J.K. Kim, J.I. Suh, C. Park, Y.C. Lee, T.W. Chung, E.Y. Song, and C.H. Kim. 2001. Characterization of a cell line HFH-T2, producing viral particles, from primary human fetal hepatocytes infected with hepatitis B Virus. *J. Microbiol. Biotechnol.* 11, 186-192.

Shin, H.R. and J.B. Kim. 1994. Positivity of HBV markers and changes of HBV markers after vaccination among healthy workers. *Korean J. Epidemiol.* 16, 163-171.

Song, K.B., K.S. Choi, W.P. Lang, and J.J. Jacobson. 1999. Hepatitis B prevalence and infection control among dental health care workers in a community in South Korea. *J. Public. Health. Dent.* 59, 39-43.

World Health Organization. 1997. Hepatitis C. *Wrld. Hlth. Org. Wkly. Epidemiol. Rec.* 72, 65-69.