Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Surveillance of viral contamination of invasive medical instruments in dentistry*

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Received May 15, 2006; revision accepted July 21, 2006

Abstract: Objective: To investigate the viral contamination of invasive medical instruments in dentistry and to provide health administrative institutions with surveillance data. Methods: Sterilized samples were randomly collected from the department of dentistry to detect HBV-DNA, HCV-RNA, HIV-RNA and HBsAg. Results: Of the invasive medical instruments that were sterilized with 2% glutaraldehyde, one of the samples was positive for HBV-DNA, and another sample was positive for HBsAg. Conclusion: Though massive virus contamination of invasive medical instruments in dentistry has been reduced to a low level, the occurrence of contamination still remains.

Key words: Glutaraldehyde, Viral contamination, Invasive
doi:10.1631/jzus.2006.B0745 **Document code:** A **CLC number:** R1

INTRODUCTION

Proper sterilization of invasive instruments and equipments is of great importance in the practice of dentistry, where various blood- or saliva-borne pathogens could be easily transmitted to patients and dental staff via contaminated or inadequately sterilized instruments (Hanson *et al*., 1990; Garcia *et al*., 2000; Zhou *et al*., 2000). Although nosocomial viral infections have been recognized for years, there is little scientific data on the potential risk of viral transmission and infection in China. Given the prevalence of HBV and HCV infection and the increasing incidence of HIV infection, these viruses are of particular concern in this study. In order to investigate the viral contamination of invasive medical instruments in dentistry, we detected HBV-DNA, HBsAg, HCV-RNA and HIV-RNA on sterilized mouth mirrors, detectors, excavators, pliers, elevators, burs, dental forceps, dental scaler tips and turbine handpieces.

MATERIALS AND METHODS

Dental instruments

Seven hundred ninety-five packed and sterilized dental instruments were randomly sampled at the stomatology department of a provincial hospital and a municipal hospital. The following items were analyzed for viral contaminants: mouth mirrors, detectors, excavators, pliers, elevators, burs, dental forceps, dental scaler tips and turbine handpieces. The instruments fell into two groups. Group A included instruments sterilized by autoclaving involving pre-vacuum steaming at 134 °C for 2 min. Group B included instruments sterilized by immersion in 2% glutaraldehyde.

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* Project (Nos. 021103128 and 2003C13015) supported by the Department of Science and Technology of Zhejiang Province, China

Samples collection

A sterile swab, immersed in with 1 ml sterile PBS, was used to wipe the instrument surface ten times. Both the swab and rinse sample were collected in a sterile 1.5 ml micro-centrifuge tube, previously dipped in 0.1% DEPC for 2 h and sterilized by autoclaving (Zhang, 2004; Zhou and Xu, 2003). Rinse samples were sent immediately to the Institute of Infectious Diseases of Zhejiang University for further viral analysis.

Processing and conservation

Rinse solution was divided into three tubes: one was mixed with Trizol (Gibco products, BRL) (1:10) and stored at −70 °C for HCV-RNA and HIV-RNA detection; one was directly stored at −20 °C for HBV-DNA and HBsAg detection; and the third one was stored at −70 °C in case further confirmatory tests were required.

Viral detection

The following steps for the investigation were submitted to the clinical laboratory of our institute. The detections of HCV-RNA and HIV-RNA were performed using nested RT-PCR. RNA was extracted according to the Trizol method as follows: Samples were thawed, homogenized and incubated for 5 min at room temperature in 1.5 ml Eppendorf tubes. Chloroform (0.2 ml) was added to each sample. The tubes were shaken vigorously by hand for 15 s and then incubated at room temperature for 2 min. Samples were centrifuged at 12000×g for 15 min at 4 °C. Sixty percent volume of the aqueous phase was transferred to a fresh tube. RNA was precipitated from the aqueous phase by mixing with 0.5 ml isopropyl alcohol. The samples were incubated at room temperature for 10 min and centrifuged at 12000×g for 10 min at 4 °C. The supernatant was decanted; 1 ml of 75% ethanol was added to each sample which was then centrifuged at 7500×g for 5 min at 4 °C. The RNA pellet was briefly dried and 20 μl DEPC-treated sterile deionized water was added. Ten microlitres solution was set aside for HCV-RNA and HIV-RNA detection, and the remaining 10 μl was stored at −70 °C in case confirmatory tests were needed. The first step, reverse transcription, was carried out using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas), a genetically engineered version of the M-MuLV reverse transcriptase. The reaction was carried out according to the enzyme instructions. Nested PCR primers and methods were based on the papers (Heermann *et al*., 1996; Hu *et al*., 2000; Nedjar *et al*., 1991; Wang *et al*., 2002). The primers were as follows: HIV1 outer-f 5′>tac agg agc aga tga tac ag<3′, HIV1 outer-r 5′>cct ggc ttt aat ttt act gc<3′; HIV1 inner-f 5′>gga aac caa aaa gat agg g<3′, HIV1 inner-r 5′>att atg ttg aca ggt gta gg<3′. HCV outer-f 5′>ctc ccc tgt gag gaa cta ctg tct t<3′, HCV outer-r 5′>ggt gca cgg tct tac gag acc t<3′; HCV inner-f 5′>tct agc cat ggc gtt agt atg gag tgt<3′, HCV inner-r 5′>ctc gca agc acc cta tca gg<3′. The PCR was carried out using Pyrobest™ DNA Polymerase (TAKARA). Both assays were conducted on a thermocyling machine (Thermo Hybaid PX2). Each reaction was overlaid with mineral oil (Sigma). PCR parameters were: 1 cycle of 95 °C for 3 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s; 1 cycle of 72 °C for 7 min with the procedure for the first and second round PCR being the same. Detection of HBV-DNA was performed by fluorescent quantitative PCR using a Shanghai Fuxing reagent. HBsAg was quantified by radioimmunoassay (RIA) using WeiFang 3V products.

RESULTS

The results of surveillance are shown in Tables 1 and 2. Group A: Out of a total of 430 instruments in the group, there were two positive results, one positive PCR signal for a bur and one positive RIA for a turbine handpiece. Group B: No positive results were detected from the 365 dental instruments.

DISCUSSION

Glutaraldehyde, a 2% aqueous solution, is commonly used as a cold and cost-effective sterilizer with potent, broad-spectrum and low-corrosivity characteristics. Currently it is being widely employed in dental laboratories and theaters of Chinese hospitals, especially in basic hospitals. However, evidence shows that glutaraldehyde is unstable, and that the effective concentration of the solution gradually decreases with time through several pathways, including

Table 1 The results of dental instruments sterilized by immersion in 2% glutaraldehyde

Instruments	Number of samples	HBsAg		HBV-DNA		HCV-RNA		HIV-RNA	
		Positive	Positive ratio (%)	Positive	Positive ratio (%)	Positive	Positive ratio (%)	Positive	Positive ratio (%)
Burs	65	0	0	1	1.54	0	0	0	0
Dental forceps	50	0	0	0	0	0	0	0	0
Excavators	50	0	0	0	0	0	0	0	0
Detector	50	0	0	0	0	0	0	0	0
Elevators	50	0	0	0	0	0	0	0	0
Dental scaler tips	50	0	0	0	0	0	0	0	0
Turbine handpieces	65	1	1.54	0	0	0	0	0	0
Mouth mirror	50	0	0	0	0	0	0	0	0

Table 2 The results of dental instruments sterilized by autoclaving

Instruments	Number of samples	HBsAg		HBV-DNA		HCV-RNA		HIV-RNA	
		Positive	Positive ratio (%)	Positive	Positive ratio (%)	Positive	Positive ratio (%)	Positive	Positive ratio (%)
Burs	50	0	0	0	0	0	0	0	0
Dental forceps	50	0	0	0	0	0	0	0	0
Excavators	50	0	0	0	0	0	0	0	0
Pliers	50	0	0	0	0	0	0	0	0
Detector	50	0	0	0	0	0	0	0	0
Elevators	50	0	0	0	0	0	0	0	0
Turbine handpieces	65	0	0	0	0	0	0	0	0

hydrolysis, decomposition, and coagulation. Meanwhile, microbiological contamination occurs in this solution (Dai *et al*., 2004). It is suggested that there is an inverse relationship between germicidal activity and reuse of the chemical sterilizing agent with time. Huying's survey reported that 2% glutaraldehyde showed excellent germicidal activity against all the test organisms within the reuse period of 4 d, although the efficacy dropped and microbial contaminants accumulated in the following days, especially after 7 d (Hu, 2002). In our study, we tested 430 dental instruments sterilized with 2% glutaraldehyde. From the sample number, one HBV DNA positive (PCR analysis) specimen out of 65 burs and one HBsAg positive (RIA analysis) specimen out of 65 handpieces were found.

Regarding various methods for sterilizing dental instruments, pre-vacuum steam autoclaving is ideal and reasonable for items that are not sensitive to heat and moisture. Pre-vacuum sterilizers are fitted with a pump to create a vacuum in the chamber to allow faster and more positive steam penetration throughout the entire load. This method is considered to be highly effective as long as it is operated correctly. Our study has substantiated previous findings and also showed no contaminated items in all the 365 samples analyzed. Apparently, routine dental services are quite affordable under normal circumstances, although expensive dental equipment, high cost of equipment maintenance and damage inflicted on dental instruments during the course of steam sterilization make them prohibitively expensive. Due to the heavy economic burden associated with dental services, the wide installation and use of dental equipment in general hospitals has been drastically curtailed and this has greatly marginalized the access to dental care.

Although our study showed that viral contamination of invasive dental instruments may not have been truly reflected in some health institutions due to strictly controlled access to medical data, we still noticed the following problems in the decontamination process: (1) The instructions for sterilization and disinfection were not always strictly followed. An enormous number of patients seeking daily medical attention, a low doctor to patient ratio, limited medical equipment and the lack of technical knowledge on

the decontamination processes are factors that result in the inadequate sterilization of dental instruments and hence intensify the risk of transmission of microorganisms. (2) The use of 2% glutaraldehyde beyond its expiration. (3) The dearth of measures to prevent sterile instrument recontamination. (4) Too much attention being paid to nosocomial infection caused by bacteria rather than virus.

Preclusion of dental instrument-borne viruses through sterilization is of paramount importance in reducing chronic blood-borne and life-threatening infections such as HBV, HCV and HIV. In mainland China, chronic HBV carrier state constitutes more than 10% of the entire population, the rate being the highest in the entire world; with the morbidity of HCV being over 3%. The HIV occurrence rate has been gradually increasing over the past twenty years. The greatest medical challenge facing the nation at the moment is to devise viable mechanisms to contain and manage these viral infections, with this issue becoming a hot topic in medical, social and political circles.

In developed countries, controlling nosocomial virus infections is part of an indispensable routine of any health care unit. In China, however, measures for controlling virus infection focus mostly on the risk of transmission of blood borne pathogens. By following steps such as strict testing of blood donors, blood, and blood products for bacterial and viral infectious agents and enforcement of strict supervision of blood banks, nosocomial infections have been tremendously minimized. In clinical institutions, endoscopes and many dental instruments may come into contact with patients' body fluids such as saliva, blood or mucous membranes during treatment or examination. Without proper disinfection, these invasive instruments and equipment may be another important route by which viruses are transmitted from patients to dental staff and vice versa.

Generally in a clinical setting, the patients subjected to instrument-related invasive treatment are far more numerous than blood donors and recipients, therefore, the risk of the spread of infections through the use of inadequately sterilized instruments is much higher than that by blood transfusion. Thus, it is extremely important to prevent the spread of infectious diseases by blocking the routes of the virus transmission via invasive diagnosis and medical instrument-related treatments.

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