Efficacy of Four Disinfectants Against Hepatitis B Virus

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Abstract

Background and Aim: Hepatitis B virus (HBV) is an important infectious agent in dentistry necessitating the use of disinfectants to prevent its transmission. This study compared the efficacy of 2/100 sodium hypochlorite disinfectants prepared from 5% solution manufactured by four different manufacturers namely Ashimashi, Paknaz, Vitex and Active for disinfection of surfaces infected with HBV.

Materials and Methods: In this in vitro experimental study, sera of 10 hepatitis B patients were poured into microtubes and 2/100 sodium hypochlorite solutions prepared from 5% Ashimashi, Paknaz, Vitex and Active disinfectants were added to them in 1/1 ratio. Polymerase chain reaction (PCR) was performed using virus diagnostic kits to detect the viral genome. Real-time PCR was performed before and after incubation with the disinfectants to assess the viral load in the serum. The reduction in the viral load of HBV was statistically analyzed by Kruskal-Wallis and Mann-Whitney U tests.

Results: No significant antiviral efficacy was noted following the application of Ashimashi 2/100 sodium hypochlorite disinfectant. Paknaz showed the highest efficacy against HBV. Vitex and Active ranked next with significant differences (p<0.0001)

Conclusion: Under the study limitations, Paknaz 2/100 sodium hypochlorite solution was the most effective while Ashimashi 2/100 sodium hypochlorite disinfectant did not show adequate efficacy against HBV.

Key Words: Hepatitis B, Disinfection, Surface, Dentistry

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Introduction

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Hepatitis B virus is among the main causes of morbidity, mortality and disability worldwide. This virus is highly resistant and remains viable in a dry blood clot for 3-4 weeks. During this time period, the virus may be indirectly transmitted from the infected objects/instruments to healthy individuals [1]. Surfaces infected with HBV may explain disease transmission in case of absence of direct cutaneous or mucosal contacts [2]. Since some of the surfaces and countertops are not sterilizable, disinfectants must be necessarily used. Several disinfectants are used for this purpose. The current study focuses on sodium hypochlorite solutions available in the market. Sodium hypochlorite is among the most commonly

used compounds for chemical disinfection due to high antimicrobial activity, not leaving toxic residues, easy application and cost-effectiveness. Sodium hypochlorite is available in liquid form in an original concentration of 5.25%. The Center for Disease control (CDC) in the United States has recommended the use of 500-5000 ppm sodium hypochlorite as an effective agent for elimination of HBV. It has been shown that 0.1% concentration of this solution can deactivate HBV

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after 10 minutes [3]. Disinfection can be done by immersion in disinfectants or use of spray and foams [4]. However, the efficacy of each of these methods depends on several factors such as number and type of infective microorganisms, concentration of the chemical agent, duration of contact with the chemical agent and the amount of blood or saliva on the instruments [5].

All sterilization methods eliminate HBV; however, it is resistant to UV radiation, ether and alcohol [6]. On the other hand, not being able to culture HBV in vitro limits studies on the efficacy of different disinfectants. For this reason, researchers have to use animal models or methods such as PCR. In the recent years, some concerns have been raised about the efficacy of some disinfecting agents against HBV [7].

Ito et al. evaluated the effects of ethanol on antigenicity of HBV envelope proteins. In specimens treated with 1% sodium hypochlorite no sign of viral DNA was detected, but viral DNA remainedin all specimens treated with ethanol [8]. Roberts et al. assessed the effects of ortho phthalaldehyde (OPA) on HBV and hepatitis C virus (HCV) infections and concluded that OPA was effective on HBV and HCV [9].

Arami et al. evaluated the effects of three disinfectants on HBV (1/100)and 1/10concentrations of 5% sodium hypochlorite and 50AF Deconex). The results showed that in all specimens treated with 1/10 concentration of 5% sodium hypochlorite solution viral DNA had been destroyed; but among specimens treated with 1/100 sodium hypochlorite, viral DNA remained in one sample. In specimens disinfected with 50AF Deconex, viral DNA was not eliminated in four samples [10]. Actual efficacy of a disinfecting agent is important because the manufacturers sometimes over-estimate the efficacy of their products. This study compared the efficacy of 2/100 sodium hypochlorite disinfectants prepared from 5% solutions manufactured by four different manufacturers namely Ashimashi, Paknaz, Vitex and Active for disinfection of surfaces infected with HBV.

Materials and Methods

In this in-vitro experimental study, 10 HBVinfected serum samples with different concentrations were obtained from the Pasteur Institute of Iran and poured into microtubes. Disinfectants including 2/100 concentration of sodium hypochlorite prepared from 5% solutions of Ashimashi, Paknaz, Vitex and Active were added to the microtubes in 1/1 ratio. After waiting for a period of time recommended by the manufacturer, PCR was performed. Tap water was used as the negative control group and 5% Paknaz sodium hypochlorite solution (YasChemi, Iran) was used as the positive control group. For conduction of the tests, first the viral load in the serum of patients was calculated using Real-time PCR kit (Genome Diagnostics, Germany, Berlin) [11] and after incubation of the samples with the disinfectants, the viral load was calculated again in order to quantitatively assess the efficacy of disinfectants. After conduction of PCR and preparing four microtubes for each serum sample, data for all four disinfectants were collected. The reduction in HBV load was analyzed using the Kruskal Wallis test and the Mann Whitney U test. The Bonferroni-Dunn adjustment was used for

The Bonterroni-Dunn adjustment was used for pairwise comparisons of disinfectants.

Results

No specific antiviral efficacy was noted in application of 2/100 concentration of Ashimashi sodium hypochlorite. Among the remaining three solutions, Paknaz had the highest efficacy and Vitex and Active ranked next with significant differences (p<0.0001) (Table 1). Non-parametric Kruskal Wallis test showed significant differences among the four disinfectants in terms of reduction of HBV load (p<0.0001). Mann Whitney U test also showed significant differences between Paknaz and Active (p<0.0001), Paknaz and Vitex (p<0.0001) and Active and Vitex (p<0.002).

Discussion

The HBV and HCV are major threats to the human community. There are 350 million HBV chronic carriers and 170 million HCV chronic carriers worldwide. Most of these patients are at risk of hepatic cirrhosis and hepatocellular carcinoma.

Moreover, the best method to confront HBV is disinfection, decreasing its risk of transmission and breaking its chain of infection in dental offices and clinics [9].

Disinfectant	Number	Mean percentage of reduction in viral load	Standard deviation	Minimum	Maximum
Paknaz*	10	99/99	0/00473	99/98	100/0
Active*	10	99/95	0/01945	99/91	99/97
Vitex*	10	99/98	0/01154	99/95	99/99
Ashimashi*	10	98/55	0/078	98/40	98/73

 Table 1. The mean and standard deviation of reduction in HBV load following exposure to different sodium hypochlorite solutions

Although HBV has shown resistance to someagents, it is not highly resistant to disinfectants in general, and strong disinfectants such as 2% glutaraldehyde aqueous solution can efficiently eliminate it [7].

Based on the results of the current study, 2/100 concentration of 5.25% Ashimashi sodium hypochlorite solution had poor efficacy against HBV. Paknaz sodium hypochlorite solution showed the highest antiviral efficacy and Vitex and Active sodium hypochlorite solutions were also effective against HBV. Considering the fact that HBV can be transmitted in amounts as small as 100,000 viruses/mm [12], only 2/100 concentration of Paknaz 5.25% sodium hypochlorite solution can be used for surface disinfection according to the CDC recommendations. Although Vitex and Active also significantly decreased the viral count, considering the high transmission rate of HBV and that itremains viable in many materials, these disinfectants cannot be reliably used to prevent disease transmission.

Sodium hypochlorite has been reported to be acceptable for chemical disinfection of surfaces infected with HBV. A study on the efficacy of disinfectants to deactivate the DNA polymerase of HBV reported that sodium hypochlorite solutions containing 2500ppm chlorine or more clearly deactivated the DNA polymerase of HBV [13].

Leontiou et al. evaluated the disinfection of diamond burs infected with HBV and showed that chlorine-containing TBS disinfecting agents had the highest disinfection efficacy against this virus [14]. Van Engelenburg et al. evaluated the spec trum of antiviral activity of an alcoholic mixture at

high concentration and reported that disinfection with alcohol at high concentrations had strong virucidal efficacy against blood-borne viruses [15]. Roberts et al. evaluated the effects of OPA, a strong disinfecting agent, on human HBV and HCV using surrogate animal viruses namely the duck HBV (DHBV) and bovine viral diarrhea virus (BVDV). They reported that OPA was effective against surrogate animal viruses [9]. Arami et al. evaluated the disinfecting efficacy of 1/100 and 1/10 concentrations of 5% sodium hypochlorite solution and 50AF Deconex and showed destrucviral DNA use tion of by of 1/10concentration of 5% sodium hypochlorite [10]. In their study, among specimens disinfected with 1/100 concentration of 5% sodium hypochlorite, decontamination did not occur in one sample (11.1%). In another study on the effects of several disinfectants on the activity of DNA polymerase of HBV, it was shown that sodium hypochlorite containing 2500ppm chlorine deactivated the DNA polymerase in one minute and consequently the virus. These results are in accord with our findings [13]. Weber evaluated the antibacterial efficacy of disinfectants in presence and absence of blood and showed that in absence of visible blood clot, the disinfected surfaces can be with 1/100concentration of sodium hypochlorite, phenol or the quarterly ammonium compounds [16]. Their results regarding sodium hypochlorite are in accordance with our findings.

Dental clinics and offices must not be sites of infection transmission. However, medical centers have always been a source of many infections and nosocomial infections have always caused problems for patients and medical personnel [17]. Pathogenic microorganisms such as bacteria, viruses and fungi can cause infections in dental clinics and offices. Many of these agents are opportunistic and can cause diseases in susceptible hosts [18].

Thus, host susceptibility to infectious agents may vary depending on patient conditions such as immunodeficiency, use of invasive treatments or the natural body defense mechanisms. Pathogenic agents particularly the opportunistic microorganisms in normal flora of patients and other individuals may be transmitted to susceptible hosts and cause infections.

Thus, infection control in medical and dental settings with high patient turnover is very important. Infection transmission via dental equipment or instruments is also a route of transmission and different disinfecting agents have been recommended to prevent infection transmission. Despite acceptable results, it should be noted that only surfaces are disinfected. Moreover, mutations in pathogenic microorganisms over time and emergence of resistant species increase concerns about resistance to antibiotics and disinfecting agents [19]. Thus, studies are required to find new efficient disinfecting agents.

Conclusion

This study showed that only Paknaz 2% sodium hypochlorite solution acceptably prevented the transmission of HBV.

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