ABSTRACT

Hepatitis B virus is the most important infectious hazard in dental profession. Vectors of HBV infection are not only blood, but also saliva, nasopharyngeal secretions, crevicular fluid.

The aim of the study was to evaluate serum and salivary HBV DNA levels in subjects with chronic infection and dynamics of HBV levels during the first 3 months of peginterferon α-2a therapy to be determined.

Nineteen parallel samples were tested for HBV DNA by real-time PCR assay.

All nineteen sera were positive for HBV DNA with levels ranging from 494 to 6,300,000,000 cp/ml. HBV DNA was detected in all saliva samples even in patients with very low viremia. HBV DNA levels in serum and saliva were quite similar in cases with serum HBV DNA < 10,000 cp/ml. Patients with viremia higher than 10,000 cp/ml had significantly lower HBV DNA levels in saliva.

The presence of HBV DNA in saliva might not be only due to transudation or exudation of fluid containing virus from the general circulation into various body fluids. These facts clearly demonstrate the role of saliva in routes of HBV transmission. Our results confirm the possibility of dentist's participiation in infection transmission and suggests that salivary analysis holds promise as a non-invasive approach to identify biomarkers for diseases.

Keywords: HBV DNA, saliva, peginterferon

INTRODUCTION

Hepatitis B virus (HBV) is the most important infectious occupational hazard in dental profession. A number of reports suggest a significant higher incidence of HBV among dental staff, especially surgeons, endodontists and periodontists [1, 2]. Vector of infection in dental practice is not only blood, but also saliva, nasopharyngeal secretions and crevicular fluid [3]. So dentists are at a high risk to spread HBV infection due to the procedures and instruments of dental treatment [4] or if a bleeding during intervention occurs.

Parenteral, sexual and vertical modes of HBV transmission are well established, but the route of horizontal transmission in childhood and among household members is still unclear. HBV DNA was detected in samples of saliva, urine, seminal fluid, nasopharyngeal fluid and tears of viremic HBV-infected subjects [5-9]. The role of saliva in transmission of HBV infection is still not well defined. Saliva may be an important source of HBV-infection as HBV transmission has been demonstrated in animals by subcutaneous inoculation of infected saliva [10-12]. HBV DNA in saliva was found at much lower concentrations than in serum, but this was described mainly in active HBV carriers with serum HBV DNA > 10,000 cp/ml [5, 7-9]. Data concerning HBV DNA levels in saliva measured by high sensitive real-time PCR assay are still limited and HBV DNA levels in saliva during therapy have not been evaluated yet.

The aim of present study was to measure the salivary HBV DNA levels in subjects with chronic HBV-infection who were with different viremia as well as to evaluate the serum and salivary levels of HBV DNA during the first 3 months of peginterferon α-2a therapy.
asked to refrain from eating, drinking, smoking for at least 1 hour before sampling. The last oral hygienic procedure had been accomplished in the previous night. Whole unstimulated salivary probe was collected as described by Dawes and Weatherell [13].

Samples were obtained from patients with chronic HBV infection who had wide-broad spectrum of serum HBV DNA level varied from hundreds to milliards copies per milliliter. One of the tested subjects was with occult HBV infection. She was HBsAg and anti-HBs negative, but anti-HBc total positive with detectable serum HBV DNA.

In five patients treatment with peginterferon α-2a was initiated, so serum and saliva levels of HBV DNA were further evaluated during the first 3 months of therapy. All were males: 4 - with chronic hepatitis B and 1 - with Child-A liver cirrhosis. Four subjects were HBeAg (-) and only one was HBeAg (+). Again parallel serum and saliva samples were obtained at baseline and at treatment days 7, 14, 21, 28 as well as 3-month after treatment initiation.

RESULTS

All nineteen sera were positive for HBV DNA with levels ranging from 494 to 6 300 000 000 cp/ml. HBV DNA was detected in all saliva samples even in patients with very low viremia. Saliva of only subject with occult HBV infection was also HBV DNA positive.

HBV DNA levels in serum and saliva were quite similar in cases with serum HBV DNA < 10 000 cp/ml (Fig. 1).

![Fig. 1. HBV DNA levels in serum and saliva in subjects with low viral load](image1)

Patients with viremia higher than 10 000 cp/ml had significantly lower HBV DNA levels in saliva (Fig. 2).

Median baseline HBV DNA level of five subjects treated with peginterferon α-2a was higher in serum then in saliva: 5 523 000 cp/ml vs. 8 357 cp/ml, respectively. A strong correlation ($r = 0.94$) was found between the median serum and saliva HBV DNA levels during the first 3 months of therapy (Fig. 3).
At the third treatment month 4 out of 5 patients were with early virological response, defined as decrease of serum HBV DNA > 1 log(10) than the baseline level. In all of them HBV DNA levels in saliva decreased with > 1 log(10), too. In addition 3/4 of early responders showed a rapid decline of both serum and saliva HBV DNA at the treatment day-28. HBV DNA in saliva remained even higher than baseline level in the non-responder.

All subjects remained viremic during the 3rd month treatment period and 3 of them were with persistently detectable HBV DNA levels in saliva, too. Only one patient cleared HBV DNA from saliva at the 3rd month after treatment initiation. HBV DNA in saliva rapidly decreased to undetectable level in another subject, but at month 3 he was again HBV DNA-positive both in serum and saliva.

**DISCUSSION**

With the advances in molecular techniques, HBV can be detected in patients with HBV infection in peripheral blood mononuclear cells, tissues of the pancreas, spleen, skin, and kidney, and fluids such as saliva, semen, vaginal secretions, sweat, breast milk, tears, and urine [14]. Although HBV DNA has been detected in wide variety of body fluids, only serum, semen and saliva have been demonstrated to be infectious [10-12]. No infections have been demonstrated in susceptible persons orally exposed to HBV-infected saliva with intact oral mucosa [10]. However, HBV transmission was described in animals after subcutaneous injection of infected saliva [11, 12]. In addition recent case-report showed that acute hepatitis B can developed after human bite by chronic HBV carrier. HBV DNA was present in the saliva of attacker. Further analysis revealed that HBV in both subjects had identical genotype and sequence [15]. Together all these findings clearly suggest that HBV transmission can occur after exposure to infected saliva of subject with skin and oral mucous lesions or damage.

The presence of HBV DNA in saliva might not be only due to transudation or exudation of fluid containing virus from the general circulation into various body fluids. Recent study reported that HBV in saliva might originate from the infected salivary glands as HBsAg, HBeAg and HBV DNA were detected in parotid cells with positive rates of 45.5%; 40.9% and 54.5%, respectively [14].

Previous study reported that 88% of viremic subjects were with detectable HBV DNA in saliva, and this was proven even by using non-sensitive assay [5]. It was also reported that HBV DNA presented at much lower concentration in saliva than in serum [5, 7-9]. By high sensitive real-time PCR technique we found HBV DNA in saliva of all tested patients both with high and very low viremia load, and even in a subject with occult HBV infection.

Our results confirm the possibility of HBV transmission during dental treatment of patients even with very low viremia. In fact dentists are at risk both to acquire and to spread HBV infection.

These data suggests that salivary analysis is a promising non-invasive approach to identify biomarkers for human diseases, including HBV infection.

**CONCLUSION**

Evaluation of HBV DNA in saliva during the first 3 months of peginterferon α-2a therapy revealed that significantly high HBV DNA level may persist in saliva within 3 months of therapy even in subjects with early virological response. To our knowledge this is the first report of parallel evaluation of HBV DNA levels in saliva and serum during therapy.

Our findings illustrate not only the diagnostic value of saliva but also it monitoring capacity during therapy. We suggest that saliva may play an important epidemiological role as a potential vehicle of infectivity.

**Conflict of interest**

Authors declare no conflict of interests.
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