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# Detection of hepatitis B surface antigen, hepatitis B core antigen, and hepatitis B virus DNA in parotid tissues

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Received 11 January 2008; received in revised form 6 March 2008; accepted 12 March 2008

Corresponding Editor: Sunit K. Singh, Hyderabad, India

## KEYWORDS

Parotid tissue;  
HBsAg;  
HBcAg;  
HBV DNA

## Summary

**Objective:** To examine the presence of hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B virus (HBV) DNA in parotid tissues from patients with positive serum HBV markers.

**Methods:** HBsAg and HBcAg were examined in parotid biopsy tissues from patients with suspected parotid tumor and positive serum HBV markers by immunocytochemistry, and HBV DNA was detected in parotid tissues by PCR.

**Results:** Among the 22 patients with a parotid tumor, only one was pathologically confirmed as a neoplasm; all others were benign. HBsAg and HBcAg were present in parotid cells with positive rates of 45.5% (10/22) and 40.9% (9/22), respectively, with an overall positive rate of 54.5% (12/22). Of the 22 cases with serum markers of HBV infection, seven (31.8%) had both HBsAg and HBcAg in the parotid cells. HBV DNA was present in seven of the 12 samples in which hepatitis B antigen was detected (58.3%).

**Conclusions:** HBV in saliva might originate from the infected salivary glands and the infectious saliva could transmit HBV.

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## Introduction

China has a high incidence of hepatitis B infection. The positive rate of serum hepatitis B surface antigen (HBsAg) is about 10–15%,<sup>1,2</sup> which translates into 120–180 million active infections in the country. Hepatitis B virus (HBV) can cause a range of human disorders such as acute hepatitis, chronic hepatitis, hepatocirrhosis, and hepatocellular carcinoma.<sup>3,4</sup> HBV can cause extrahepatic pathological lesions. 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.<sup>5–7</sup>

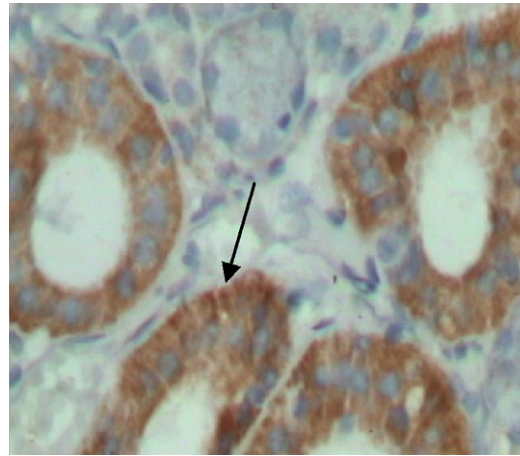
Although there have been some studies on the infection and location of HBV in extrahepatic tissues,<sup>8</sup> it remains controversial whether HBV can replicate outside the liver after acute infection.<sup>6</sup> The results of studies into the location and distribution of HBV in extrahepatic tissues or cells are not consistent, and the pathologic lesions caused by extrahepatic infection with HBV and their pathogenesis are not fully understood. Little is known about whether HBV exists in parotid tissues. If we can demonstrate that HBV exists in parotid tissues, then it is reasonable to assume that the viruses present in saliva might originate from the salivary glands. The objective of this study was the detection of HBsAg, hepatitis B core antigen (HBCAg), and HBV DNA in parotid biopsy specimens from patients with positive HBV serum markers, using immunocytochemistry and polymerase chain reaction (PCR).

## Materials and methods

We reviewed the 1992–2003 pathology registry of Xiangya Hospital, Central South University and identified all patients who had had a parotid biopsy. Because it is impossible to obtain parotid tissues from normal individuals, we used the parotid tissues taken from patients with suspected parotid tumors for pathological examination. We further selected the patients with positive serum HBsAg. Paraffin-embedded parotid tissues from the patients with positive serum HBsAg were used for laboratory investigations.

The presence of HBsAg and HBCAg in parotid biopsy tissues was determined by immunocytochemistry (streptavidin–peroxidase–biotin) according to the protocol provided by the manufacturer. Reagents used were purchased from Zhongshan Biological Technology Ltd, Beijing.

For those patients with HBsAg and HBCAg detected in the parotid tissues, HBV DNA was examined by PCR. Paraffin-embedded parotid tissues were digested with proteinase K, and DNA was isolated by phenol and chloroform. Primer sequences were as follows: forward primer, 5'-TCGGAAATACA-CCTCCTTCCATGG-3'; reverse primer, 5'-GCCTCAAGGTCGGTCGTTGACA-3'. The length of the PCR product was 350 bp. Thirty cycles of DNA amplification were performed in 50  $\mu$ l PCR reaction mixture. The conditions for each cycle were: denaturing at 94 °C for 30 s, primer annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final 10-min elongation at 72 °C. DNA marker, proteinase K, Taq DNA polymerase, and dNTP were purchased from TaKaRa Co., Dalian. Primer was synthesized by BioAsia Co., Shanghai.



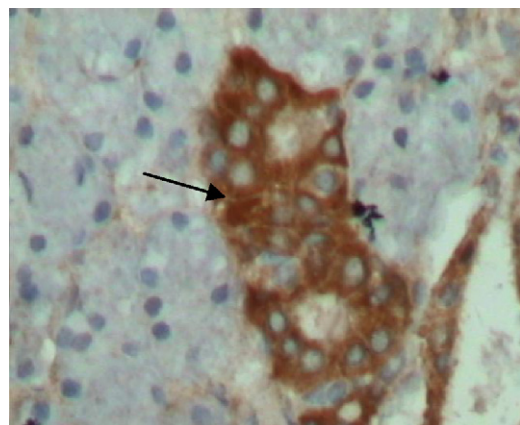
**Figure 1** Detection of HBsAg in parotid tissue by immunocytochemistry (brown particles represent positive HBsAg expression).

This study was approved by the research ethics board of Central South University.

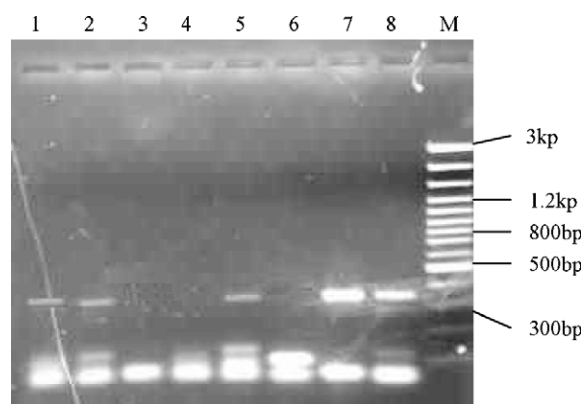
## Results

A total of 232 patients who had had a parotid biopsy were identified from the pathology registry of the Xiangya Hospital, Central South University for the period 1992–2003. Among them, 22 patients had positive serum HBV markers. All of the 22 patients (11 males and 11 females) had an initial diagnosis of parotid tumor. Among them, only one was a pathologically confirmed neoplasm, and all others were benign. The age of these patients ranged from 2 months to 71 years (median 40 years). All of the 22 patients were positive for HBsAg in serum, but no other serum HBV marker was recorded in the original pathology reports.

The results of immunocytochemistry indicated that HBsAg and HBCAg were present in parotid tissues. Brown positive particles were found predominantly within the parotid cells, depositing linearly or in clusters (Figures 1 and 2). HBsAg and HBCAg were present in parotid cells with positive rates of 45.5% (10/22) and 40.9% (9/22), respectively, with an overall



**Figure 2** Detection of HBCAg in parotid tissue by immunocytochemistry (brown particles represent positive HBCAg expression).



**Figure 3** Detection of HBV DNA in parotid tissue by PCR (M DNA marker, lanes 1, 2, 5, 7, and 8 were positive samples).

positive rate of 54.5% (12/22). Of the 22 cases with serum markers of HBV infection, seven (31.8%) had both HBsAg and HBeAg in the parotid cells. Because hepatitis B antigen was detected in the parotid tissue in only 12 cases, HBV DNA was examined in parotid tissues for these 12 patients only. HBV DNA was present in seven of the 12 samples examined (58.3%) (Figure 3). Because of incomplete data, we could not analyze the relationship between parotid HBV antigen expression and serum HBV markers.

## Discussion

Transmission of HBV occurs within and between households through daily contact, and the potential transmission vehicles include saliva.<sup>9</sup> HBsAg, HBeAg, and anti-HBe are HBV markers that can be detected both in saliva and serum.<sup>10,11</sup> HBV DNA has been detected in saliva from HBsAg-positive patients.<sup>12</sup> One study has shown positive rates of HBV DNA in saliva of 64.2% in acute HBV infections and 61.6% in HBV carriers.<sup>13</sup> Another study found a high correlation between serum HBV DNA and saliva HBV DNA.<sup>14</sup> However, there is little information regarding whether the parotid gland can function as a site of HBV location and replication. The major obstacle for research aimed at providing an answer to this question is the difficulty in obtaining tissues from the salivary glands.

Our study results indicate that HBsAg, HBeAg, and HBV DNA were present in parotid tissues, which is consistent with a previously reported study.<sup>15</sup> The findings from our study and earlier ones suggest that HBV in saliva might originate from infected salivary glands. In our study, parotid biopsies were performed following a standard pathology protocol (i.e., parotid tissues were washed, antisepticized, and paraffin-embedded), so that the possibility of a false positive parotid HBV finding caused by blood contamination was very small. Although the samples in our study were taken from patients with suspected parotid tumors, only one was a pathologically confirmed neoplasm, and all others were benign cases. Even for the neoplasm case, we used normal parotid tissues for HBV examination. As a result, our finding could represent HBV infection in normal parotid tissues.

The presence of HBsAg and HBeAg in the human body suggests that the individual can make antibodies, and HBV antigen and antibody can form immune complexes. If HBsAg

and HBeAg are present in tissues, the immune complexes thus formed can cause pathological lesions in the tissues. When HBV DNA is present in the tissues, HBV can replicate and therefore establish infection. Because HBV replication can increase HBV antigens in the circulation, HBV antigens can deposit in the tissues leading to tissue immune lesions.<sup>16</sup>

The presence of antigen and DNA in saliva could decline after the acute phase. Unfortunately, our study was based on retrospective parotid tissue samples obtained from the pathology department. As a result, we could not differentiate acute from chronic infection, nor could we compare viral load in saliva with that in the parotid. Theoretically, compared with HBsAg and DNA, the presence of HBeAg represents a higher risk for passing on the infection. However, since we were not able to look at the presence of antigen in parotid gland and saliva simultaneously, we could not examine this issue.

HBV is a partially double-stranded DNA virus of the Hepadnaviridae family. The length of the HBV genome is 3.2 kb.<sup>17</sup> There are several kinds of HBV DNA at different replication stages, such as HBV covalently closed circular DNA (cccDNA), relaxed circular DNA (rcDNA), single-stranded DNA, and integrated DNA. In the lifecycle of HBV, cccDNA is a template for transcription of viral pregenomic RNA and all messenger RNA.<sup>18</sup> cccDNA is a replicative intermediate of HBV DNA, which sustains the HBV infection in human bodies<sup>19</sup> and plays an important role in HBV replication and the establishment of HBV infection. However, due to lack of funds, we did not examine cccDNA in parotid tissues.

In summary, our study based on 22 patients with positive serum HBV markers, suggests that the parotid gland can serve as a site of HBV location. Whether HBV can replicate in parotid tissues deserves further investigation. Future studies on these research questions could lead to the discovery of potentially new HBV transmission routes and could provide scientific evidence for the control and prevention of hepatitis B. For example, if HBV detected in saliva has originated in the salivary glands instead of blood, HBV transmission could be realized through intimate contact with HBV-positive individuals.

## Acknowledgments

This study received support through a grant from the Hunan Ministry of Science and Technology (No. 04SK3044-1), China. We thank the patients and staff of the participating hospitals in Changsha, China for their support.

*Conflict of interest:* No conflict of interest to declare.

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