J Oral Pathol Med (2006) 35: 626–9

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No evidence of hepatitis C virus infection in Serbian patients with oral leukoplakia

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BACKGROUND: A review of the literature reveals controversy regarding the relationship between hepatitis C virus (HCV) infection and oral leukoplakia (OL). The aim of this study was to determine the frequency of HCV antibodies in patients with OL and control subjects resident in Serbia.

METHODS: In this cross-sectional study 73 consecutive patients with histologically proven OL and 90 control subjects, whose age and gender were matched, were examined for the presence of serological evidence of chronic hepatic disease, hepatitis B surface antigen (HBsAg) and anti-HCV seropositivity.

RESULTS: None of the patients with OL or control subjects had antibodies to HCV or HBsAg. All patients with OL and control subjects had normal liver function.

CONCLUSION: The present data indicate that patients with OL resident in Serbia do not have evidence of HCV or HBV infection.

J Oral Pathol Med (2006) **35**: 626–9

Keywords: hepatitis C; virus diseases; risk factors; oral leukoplakia; hepatitis C antibodies; hepatitis B surface antigens

Introduction

The majority of subjects infected by hepatitis C virus (HCV) develop chronic liver disease, and 20% of these subjects infected over the age of 40 progress slowly to liver cirrhosis within 20 years (1, 2). Once cirrhosis is established, the risk for development of hepatocellular carcinoma (HCC) is approximately 1–4% per year (3, 4).

Aside from hepatic malignancy, HCV infection is associated with extrahepatic malignancies such as non-Hodgkin's lymphoma (5, 6). Furthermore, high prevalence of both anti-HCV antibodies and HCV-RNA in patients with oral cancer has been observed in an HCV hyperendemic area of Japan (7). The results of a large national case–control study in Japan have also supported the possible association between HCV infection and head and neck squamous cell carcinoma (SCC). It has been suggested that HCV infection may be a cofactor in the development of oral SCC (8) and anti-HCV-positive patients with oral cancer should be considered at high risk for the development of multiple primary neoplasms and should be closely monitored (9). HCV replication has been reported in the oral cancer tissue only in subjects with HCV-RNA-positive serum (10). Some studies have shown that both positive and negative HCV-RNA strands are detectable in healthy oral mucosa tissue of anti-HCV-positive subjects (10, 11).

A significantly high incidence of oral leukoplakia (OL) has been found among anti-HCV and HCV-RNA-positive subjects from northern Kyushu region in Japan, where the prevalence of HCV infection is the highest in the country (12). However, studies from the UK have failed to demonstrate a significant association between HCV and oral epithelial dysplasia (13).

Thus, there seems to be controversy with respect to the relationship between HCV infection and OL. The aim of this study was to determine the frequency of HCV antibodies in patients with OL and compare it with that of control subjects.

Patients and methods

Sample size was calculated based on $\alpha = 0.05$ and $\beta = 0.20$, a prevalence of HCV infection in the population of 0.78% (14), an expected prevalence of HCV antibody in the OL group of 21% (12). A minimum sample size of 37 subjects for each group was obtained.

Seventy-three consecutive patients with OL referred to the Dentistry Department of the Faculty of Medicine, Novi Sad, Serbia, in the period January 2002–May 2005, were enrolled in this cross-sectional study. The diagnosis of OL was based on both clinical and histological findings (15). Leukoplakias were staged according to a proposed classification and staging system (16). The investigated group included 41 men and 32 women (mean age 49.2 years). Seventy patients (95.9%) had homogeneous leukoplakia and three (4.1%) had

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Accepted for publication June 19, 2006

non-homogeneous leukoplakia. Seventeen cases (23.2%) had stage I (L1P0); 31 cases (42.5%) had stage II (L2P0), while 23 cases (31.6%) had stage III (L3P0) and two cases (2.7%) had stage IV (L3P1).

The control group consisted of 90 individuals without oral mucosal lesions referred to the same department during the same period for reasons such as periodontal, prosthodontic and endodontic treatment. There were 53 men and 37 women, with mean age of 47.0 years. Controls were matched to the cases on gender, age (± 5 years), time of enrollment and residence. Oral biopsies were not performed on control subjects.

On admission to the study, by means of a questionnaire, a detailed medical history was taken from each subjects, including data on alcohol consumption, smoking and their potential risk for viral hepatitis. Informed written consent was obtained from each subject. The study protocol was approved by the Ethics Committee of our faculty.

The following parameters were recorded in blood analysis using standard laboratory methods: complete blood count, total bilirubin, direct bilirubin, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). In addition, all serum samples of patients and controls were tested for hepatitis B surface antigen (HBsAg) by an enzyme-linked immunosorbent assay (ELISA) (Hepanostika HBsAg Uni-Form II). Anti-HCV antibodies were detected by UBI HCV EIA 4.0 in both groups. Serum HCV RNA was not measured.

Chi-square test with Yates' correction or Fisher's exact probability test was used to compare relative frequencies between the two groups. Student's *t*-test was used to evaluate differences between means of the two groups. $P < 0.05$ was considered significant.

Results

The clinical characteristics of patients with OL and controls are shown in Table 1. No difference was found between patients with OL and the control group, regarding mean age, sex, surgical treatments, blood transfusions, hepatotoxic drugs, and tattoos.

Twenty-six patients with OL and 29 control subjects had a history of surgical procedures in the past (three patients and one control 5 years ago, five patients and seven controls 10 years ago, and 18 patients and 21 controls more than 20 years ago).

Three patients with OL, but only one of the control subjects, received blood transfusion several years ago (two patients 10 years ago, one patient 7 years ago, one control subject 5 years ago). Only three patients were at risk for viral hepatitis, due to needle-stick injuries as they were healthcare workers.

Regular alcohol consumption was more frequent in OL patients than in controls ($P < 0.05$), whereas no statistically significant difference was found for occasional alcohol consumption between these two groups.

Use of potentially hepatotoxic drugs was observed in 9.6% of patients with OL and in 5.5% of control subjects, including ranitidin (two patients), diclofenac (two patients and two control subjects), captopril (two patients and two control subjects), and fluconazole (one patient).

None of the patients or control subjects had abnormal liver function tests. In addition, there were no significant differences in mean values of serological hepatic tests between the two groups.

Hepatitis B surface antigen was not found in any patient with OL or control group subject. None of the patients with OL or control subjects were HCV seropositive.

Table 1 Clinical characteristics of patients with oral leukoplakia (OL) and controls

Characteristics	*OL (n = 73)*	*Controls (n = 90)*	*Statistical significance*
Age (years; mean ± SD)	49.2 ± 12.5	47.0 ± 10.6	NS
Sex (male/female)	41/32	53/37	NS
Surgical treatments	26	29	NS
Blood transfusions	3	1	NS
Needle-stick	3	0	NS
Tattooing	1	0	NS
Alcohol consumption			
Occasional	9	8	NS
Regular	15	0	$P < 0.001$
Hepatotoxic drugs	7	5	NS
Total bilirubin (μmol/l; mean ± SD)	11.6 ± 3.9	10.7 ± 2.3	NS
Direct bilirubin (μmol/l; mean ± SD)	2.3 ± 1.6	2.8 ± 2.1	NS
Total protein (g/l; mean ± SD)	71.5 ± 5.1	69.9 ± 6.3	NS
Albumin (g/l; mean ± SD)	42.0 ± 5.6[a]	43.0 ± 7.0	NS
ALT (U/l; mean ± SD)	18.3 ± 8.3	16.6 ± 5.2	NS
AST (U/l; mean ± SD)	21.0 ± 7.0	22.6 ± 6.4	NS
ALP (U/l; mean ± SD)	65.4 ± 20.8	68.7 ± 22.4	NS
HBsAg	0	0	
Anti-HCV	0	0	

NS, not significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.
[a]Serum albumin was available for 45 patients.

Discussion

Unsafe healthcare procedures and blood transfusion may be important means of HCV transmission. However, the present study did not find an association between HCV infection and exposure to surgical procedures and receipt of blood transfusion.

Alcohol consumption causes chronic hepatic injury, but it may be a risk factor for oral cancer and OL as well. In the present study regular alcohol consumption was found in 20.5% of patients with OL, and none of these cases had a history of alcohol intake over 20 g/day or abnormalities in liver function tests. Furthermore, liver enzyme values (ALT, AST, and ALP) did not reflect any abnormal response to liver function tests in patients with OL. On the contrary, Nagao et al. (7) reported that serum AST and ALT levels were significantly higher in 24 HCV-positive oral cancer patients than in 76 HCV-negative oral cancer patients.

This prospective study was primarily designed to examine the HCV status of patients with OL compared with controls. It is of great significance that none of the patients with OL or control subjects were HCV seropositive. This observation is in accordance with the findings of other similar studies (13). However, in an HCV hyperendemic area of Japan with the prevalence of 12.3%, Nagao et al. (12) found OL in 18 (21.4%) out of 84 cases with HCV infection. In the same study, the incidence of OL in subjects with HCV infection was significantly higher than in those without HCV (4.4%). Likewise, they found no relationship between the severity of liver dysfunction and appearance of OL. It should be noted that in the above-mentioned study, the clinical diagnosis of OL was not confirmed histologically. In this sense, all patients with OL in the present study underwent histopathological confirmation of the disease. In an analysis of 70 subjects (52 patients with histologically documented OL) Carrozzo et al. (17) reported that three cases (4.3%) were HCV seropositive, whereas Ferreiro et al. (18) found one case of OL among 74 patients with chronic HCV infection.

The apparent lack of an association between HCV infection and OL in this study parallels that between HCV and oral lichen planus in Serbian patients (19). The negative results possibly reflect the low incidence of HCV infection in the general population of Serbia. The incidence of chronic HCV infection in Serbia was 5.05/100 000 inhabitants in 2003 and 4.35/100 000 inhabitants in 2004. HCV infection was most common in the 20- to 29-year-old age group (20).

It has been suggested that the geographical origin of patients included in the study could strongly influence the results, especially if they are from countries such as Italy, Spain or Japan, where the prevalence of HCV infection is high (13). The present study has included only patients with OL and control subjects living in the northern region of Serbia (the province of Vojvodina). The potential confounding bias of demographic factors such as gender and age was excluded by study design. In addition, both groups had comparable risk factors for HCV infection.

Taken together, the findings from this study suggest that there is no evidence for an etiopathogenetic role for HCV in OL patients resident in Serbia.

A review of the literature suggests a possible association between hepatitis C and the development of oral SCC (7–9). Recently, Nobles et al. (21) found that patients positive for the anti-HCV antibody had an earlier age of onset of head and neck SCC than patients negative for the antibody. The age differences between the two populations might reflect differences in risk factors for the development of hepatitis C. Moreover, there is molecular evidence that hepatitis C might contribute to carcinogenesis (10).

In conclusion, the present data indicate that patients with OL resident in Serbia do not have an increased frequency of hepatic disease or evidence of HCV or HBV infection. These findings provide evidence against a link between HCV and OL in Serbian patients.

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Acknowledgements

This study is part of the research project No. 101490 partially financially supported by the Ministry of Science and Environmental Protection of Serbia.