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Stage of hepatocellular carcinoma is associated with periodontitis

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Abstract

Aim: Periodontitis induces overproduction of reactive oxygen species (ROS). This state increases circulating ROS levels and may affect hepatocellular carcinoma (HCC). The Japan Integrated Stage (JIS) score is a novel staging system for HCC. The objective of the present study was to compare JIS scores in HCC patients with and without periodontitis.

Material and Methods: We recruited 64 HCC patients comprising 31 chronic periodontitis subjects (HCC + P) and 33 periodontally healthy controls (HCC + H). Their JIS scores were recorded. Serum levels of reactive oxygen metabolites (ROM) from HCC + P, HCC + H and healthy age- and gender-matched subjects with healthy gingiva (control, $n = 15$) were also assessed for circulating ROS levels.

Results: The HCC + P and HCC + H groups had similar body mass index, habitual drinking and tobacco exposure data. The HCC + P group showed higher JIS scores than the HCC + H group ($p = 0.027$). Both the HCC + P and HCC + H groups had higher serum levels of ROM than controls ($p < 0.001$), while serum levels of ROM in the HCC + P group were a further 25.8% higher than those in the HCC + H group ($p < 0.001$).

Conclusion: HCC patients with periodontitis had higher JIS score and circulating ROS level than HCC patients without periodontitis.

Key words: anti-oxidant; carcinoma, hepatocellular; JIS score; oxidative stress; periodontitis

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Periodontitis is a chronic inflammatory disease of the tissues that support the teeth and is caused by subgingival plaque biofilm (Madianos et al. 2005, Komiya-Ito et al. 2010). When stimulated by bacterial pathogens, host inflammatory cells produce reactive oxygen species (ROS) as part of the immune response (Sculley & Langley-Evans 2002). However, overproduction of ROS from inflammatory cells also induces oxidation of various molecules (oxidative stress) in the periodontal tissue and this is one of the pathological features of periodontitis progression (Chapple 1997, Chapple & Matthews 2007).

Recent clinical studies have reported the effects of periodontitis on circulating ROS and oxidative stress. For instance, it is known that total oxidative status (Akalın et al. 2007, Wei et al. 2010), lipid peroxidation (Akalın et al. 2007), protein carbonyl (a marker of protein oxidation) (Baltacıoğlu et al. 2007) and reactive oxygen metabolites (ROM) (overall oxidant capacity against *N*, *N*-diethylparaphenylendiamine in acidic buffer) (Tamaki et al. 2009, D'Aiuto et al. 2010) in serum is significantly higher in periodontitis patients than in periodontally healthy subjects. Moreover, it has been shown that periodontal treatment decreases serum levels of ROM (Tamaki et al. 2009) and improves circulating pro-oxidant/anti-oxidant balance (Tamaki et al. 2011) in chronic periodontitis patients. These

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observations indicate that ROS produced in periodontal lesions diffuse into the blood stream.

Animal studies have demonstrated that the increased serum ROS following experimental periodontitis may be detrimental to hepatic health (Tomofuji et al. 2007, 2008). ROS are involved in development of various cancers, including hepatocellular carcinoma (HCC) (Severi et al. 2010). In the case of HCC, ROS are involved in the transcription and activation of a large series of cytokines and growth factors, which ultimately lead to malignant transformation (Severi et al. 2010). Therefore, it is possible that periodontitis affects HCC by increasing circulating ROS. However, the relationships among periodontitis, HCC and circulating ROS remain unclear.

The prognosis of HCC is influenced by tumour stage and the liver function reservoir. So, several new staging systems evaluating survival of HCC include tumour stage and liver function stage. The Japan Integrated Staging (JIS) system (Kudo et al. 2004), which is based on a combination of the Child-Pugh system (Pugh et al. 1973) and tumour node metastasis (TNM) classification [International Union Against Cancer (UICC) 2002], is now available. This system is accepted by many institutions in Japan because of its simplicity and validity (Kudo et al. 2004, Luo et al. 2008).

The objective of the present study was to compare JIS score in HCC patients with and without periodontitis. Serum levels of ROM were determined as a marker for circulating ROS (Tamaki et al. 2009, D'Aiuto et al. 2010). The OXY-adsorbent test was also performed to evaluate the corresponding anti-oxidative status (Tamaki et al. 2011). Furthermore, serum levels of total bilirubin and albumin were analysed to evaluate liver function (Tateishi et al. 2005).

Material and Methods

Subject recruitment

The subjects were recruited at the Clinic of Gastroenterology and Hepatology, Okayama University Hospital, from August 2008 to December 2010. Of 180 patients who had HCC, 108 patients volunteered to receive an oral examination. We excluded 44 patients who had a history of antibiotic therapy within the last 3 months ($n = 15$) or less than 10 teeth ($n = 29$). As a result, 64 HCC patients were participated in our study, and they were divided into two groups [31 patients with periodontitis (HCC + P group) and 33 periodontally healthy controls (HCC + H group)]. Patients with chronic periodontal problems had been diagnosed with severe periodontitis ($n = 11$) or moderate periodontitis ($n = 20$). Severe periodontitis patients had two or more interproximal sites with clinical attachment level (CAL) ≥ 6 mm, not on the same tooth, and one or more interproximal sites with a probing pocket depth (PPD) ≥ 5 mm, while moderate periodontitis patients had two or more interproximal sites with CAL ≥ 4 mm occurring at two or more different teeth or two or more interproximal site with a PPD ≥ 5 mm, not on the same tooth (Demmer & Papapanou 2010). The control groups had no sign of periodontitis, i.e. neither attachment loss nor obvious clinical inflammation. Furthermore, age- and sex- matched volunteers (control group, $n = 15$), who had not ever smoked nor had any systemic diseases, were also recruited to compare serum levels of ROM and anti-oxidant status with HCC subjects with and without periodontitis. The study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. After obtaining written informed consent, the patients received an interview to complete a detailed medical questionnaire by the dentists.

Measurements of periodontal parameters

Measurements of periodontal parameters were made by a single trained examiner (T. T.) after HCC diagnosis and before any substantial cancer treatment. This clinical examiner had been previously calibrated and he was blind to the HCC staging diagnosis of each patient. PPD and CAL were measured at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) per tooth using a probe (CP-11; Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). CAL was measured as the distance in mm from the cemento-enamel junction to the bottom of the gingival pocket. Sites that bled upon gentle probing were recorded and percentage of bleeding sites to total sites was calculated in each subject. Oral hygiene status was measured using the dental Plaque Index (Silness & Löe 1964).

Blood sample collection and preparation

Fasting blood samples were collected from patients at the time of HCC diagnosis and from healthy volunteers. The collected blood samples were then centrifuged at $3000 \times g$ for 5 min. to separate serum. If not immediately assayed, serum aliquots were stored at $-80°C$ until subsequent analysis. The obtained samples were used to determine serum levels of total bilirubin, albumin, ROM and total anti-oxidant capacity.

Measurements of serum total bilirubin and albumin

Serum levels of total bilirubin were determined using a kit based on the enzymatic-colorimetric method (Alfresa Pharma Co., Osaka, Japan). Serum levels of albumin were also measured using the modified bromcresol purple method (Ono et al. 2009).

Calculation of JIS score

Stage of HCC was assessed by Child-Turcotte-Pugh (Pugh et al. 1973) and TNM classification [International Union Against Cancer (UICC) 2002]. JIS score was obtained by summing up the Child-Pugh stage score (Child-Pugh stages A, B and C were allocated scores of 0, 1 and 2, respectively) and TNM score (stages I, II, III and IV were allocated scores of 0, 1, 2 and 3, respectively) (Kudo et al. 2004).

Measurement of ROM serum levels

Measurement of serum ROM levels was performed using a spectrophotometer (Diacron International, Grosseto, Italy), as reported previously (Tamaki et al. 2009). The measurement unit used was the Carratelli

unit (CARR U); it has been established that 1 CARR U corresponds to 0.08 mg/dl hydrogen peroxide.

Measurement of total serum anti-oxidant capacity

To determine total serum anti-oxidant capacity, the OXY-adsorbent test was performed using a spectrophotometer (Diacron International) (Tamaki et al. 2011). This test evaluates the capacity of serum to oppose the massive oxidative action of a hypochlorous acid (HClO) solution and total anti-oxidant capacity was expressed in terms of HClO (μmol) consumed by 1 ml of sample (μmol HClO/ml).

Statistical analysis

Power analysis and sample size were calculated using statistical software (nQuery Advisor, Statistical Solutions, Sangus, MA, USA), based on the results of JIS score (prevalence of HCC patients with JIS score 0 versus score 1–5) between HCC + P and HCC + H groups in a preliminary study or the results of serum levels of ROM between periodontitis patients and subjects with healthy gingiva in a previous study (Tamaki et al. 2009). A sample size of 29 and 8 per group was required for detection of significant differences in JIS score and ROM (80% power, two-sided 5% significance level), respectively.

Mann–Whitney *U*-test or the chi-squared test was used to compare parameters between HCC + P and HCC + H groups. Mann–Whitney *U*-test and Bonferroni's correlation were also used to evaluate differences between the HCC + P, HCC + H and control groups.

A backward, stepwise logistic regression analysis was performed to determine the optimum model for progression of JIS score ≥ 1 (comparison with JIS score 0) using the following independent variables: gender, age, BMI, habitual drinking, tobacco exposure, presence of diabetes mellitus, presence of hypertension, type of virus infection, PPD, CAL, BOP, total bilirubin and ROM. The odds ratio (OR) and 95% confidence interval (CI) were calculated and the logistic regression models were reviewed for goodness of fit and validated by means of the Hosmer-Lemeshow statistics (Saito et al. 2001, Takeuchi et al. 2010).

All analyses were performed using software (SPSS 19.0 J for Windows, SPSS Japan, Tokyo, Japan). A p-value <0.05 for Mann–Whitney *U*-test or the chi-squared test and a p-value <0.01 for Bonferroni's correlation were considered to indicate significance.

Results

The clinical characteristics of the present HCC patients are shown in Table 1. There were no statistically significant differences between the groups with regard to gender, age, body mass index, habitual drinking and tobacco exposure. The prevalence of diabetes mellitus and hypertension and types of viral infection also showed no statistically significant differences between the HCC + P and HCC + H groups.

The median PPD ($p < 0.001$), CAL ($p < 0.001$), BOP ($p < 0.001$) and Plaque Index ($p = 0.004$) for the HCC + P group were significantly higher when compared with those for the HCC + H group (Table 2). No significant difference between the groups was observed with regard to the number of teeth present.

There were no significant differences in Child-Pugh and TNM stage between the HCC + P and HCC + H groups (Table 3). However, the JIS scores for the HCC + P group were greater than those for the HCC + H group ($p < 0.05$). Serum level of total bilirubin for the HCC + P group was also higher than that for the HCC + H group ($p < 0.01$), while there was no significant differences in serum albumin concentration between the two groups (Table 4). In addition, a backward, stepwise logistic regression model showed that the increased JIS score was significantly associated with tobacco exposure (OR: 4.112; 95% CI: 1.176–14.383) ($p < 0.05$) and PPD (OR: 3.380; 95% CI: 1.034–11.052) ($p < 0.05$).

Both the HCC + P and HCC + H groups had higher serum levels of ROM ($p < 0.001$) and lower serum anti-oxidant status ($p < 0.01$) than controls (Table 5). Furthermore, serum levels of ROM for the HCC + P group were 25.8% higher than those for the HCC + H group ($p < 0.001$) (Table 5). However, there was no significant difference in serum anti-oxidant status between the HCC + P and HCC + H groups.

Discussion

In the present study, HCC patients with chronic periodontitis showed greater JIS scores and higher serum levels of total bilirubin than those with periodontally healthy gingiva and a backward, stepwise logistic regression model showed that the progression of JIS score was significantly associated with PPD. Increased serum levels of ROM were also seen in HCC patients with chronic periodontitis when compared with those with periodontally healthy gingiva. ROM is considered to be a reliable parameter of circulating ROS (Tamaki et al. 2008, 2009, D'Aiuto et al. 2010) and it has been reported that ROS induces progression of HCC (Severi et al. 2010). The results suggest that increased circulating ROS following

Table 1. Clinical characteristics of the present subjects

Characteristics	HCC + P (n = 31)	HCC + H (n = 33)	p-value
Gender [male, n (%)]	22 (71)	25 (76)	0.779*
Age [years, median (25%, 75%)]	68 (61, 74)	69 (59, 72)	0.877†
BMI [median (25%, 75%)]	22.7 (20.4, 24.6)	23.3 (21.5, 24.8)	0.361†
Habitual drinking [yes, n (%)]	11 (35)	16 (48)	0.461*
Tobacco exposure [yes, n (%)]	12 (39)	15 (45)	0.621*
Diabetes mellitus [yes, n (%)]	5 (16)	4 (12)	0.729*
Hypertension [yes, n (%)]	5 (16)	5 (15)	1.000*
History of hepatitis			
Hepatitis B	8 (26)	8 (24)	0.864*
Hepatitis C	22 (71)	23 (70)	
Others (autoimmune hepatitis)	1 (3)	2 (6)	

*Chi-square test.
†Mann–Whitney *U*-test.

Table 2. Periodontal parameters of hepatocellular carcinoma patients with and without periodontitis

Parameters	HCC + P (n = 31)	HCC + H (n = 33)	p-value*
Number of teeth present [median (25%, 75%)]	24 (16, 27)	26 (22, 28)	0.080
Full-mouth PPD (mm) [median (25%, 75%)]	2.2 (2.0, 2.7)	1.9 (1.5, 2.1)	<0.001
Full-mouth CAL (mm) [median (25%, 75%)]	2.8 (2.6, 3.3)	1.9 (1.5, 2.1)	<0.001
CAL 4–5 mm (%)	40 (30, 51)	–	–
CAL $\geq$ 6 mm (%)	8 (4, 18)	–	–
BOP (%) [median (25%, 75%)]	30 (19, 41)	5 (2, 11)	<0.001
Plaque Index [median (25%, 75%)]	0.9 (0.6, 1.2)	0.6 (0.4, 0.8)	0.004

*Mann–Whitney U-test.

Table 3. Cancer stage and JIS score of hepatocellular carcinoma patients with and without periodontitis

Parameters	HCC + P (n = 31)	HCC + H (n = 33)	p-value*
Child-Pugh stage (n)	–	–	0.540
A	21	23	–
B	10	10	–
C	0	0	–
TNM stage (n)	–	–	0.309
I	7	14	–
II	12	7	–
III	7	7	–
IV	5	5	–
JIS Score (n)	–	–	0.027
0	5	13	–
1	9	5	–
2	9	8	–
3	8	3	–
4	0	4	–
5	0	0	–

*Chi-square test.

periodontitis may be involved in progression of HCC.

Several staging systems, including the JIS score, the Cancer of Liver Italian Program (CLIP) score and the Barcelona Clinic Liver Cancer (BCLC) staging classification, have been developed to classify patients with HCC (Chung et al. 2007). It was demonstrated that the JIS score has better stratification ability and prognostic predictive power than the CLIP score in a large cohort of Japanese HCC patients (Kudo et al. 2004). It is also known that JIS score is most suitable among these three prognostic systems (JIS score, CLIP score and BCLC staging classification) for prediction of patients with HCC diagnosed after 1990, when the frequency of early detection of HCC increased markedly (Toyoda et al. 2005). Therefore, we used JIS score for HCC classification in this study. However, the JIS score has been validated in only the Asia-Pacific region (Marrero et al. 2010). The other scoring system should be used to investigate the relationship between periodontitis and HCC in a Western population.

The present findings showed no relationship between HCC patients with and without periodontitis with regard to the Child-Pugh and TNM classifications. Child-Pugh classification only addresses the functional capacity of the liver without tumour parameters (Pugh et al. 1973). In contrast, TNM classification uses only tumour-related parameters (irrespective of functional liver capacity) for identification of HCC candidates for treatment [International Union Against Cancer (UICC) 2002]. Thus, the sensitivity of these two classifications may be too low to reveal significant differences between HCC patients with and without periodontitis.

In this study, all HCC patients had higher serum levels of ROM than control subjects. These observations indicate that HCC induce increases in blood ROS and this is consistent with previous reports demonstrating increased serum levels of malondialdehyde (index of lipid peroxidation) (Tsai et al. 2009) and ROM (Inokuma et al. 2009) in HCC patients. Furthermore, there was an association between periodontitis and elevated serum level of ROM in the present HCC patients. It is feasible that periodontitis and HCC have additive effects on elevated circulating ROS and that such responses will further heighten the chronic liver damage in HCC patients.

On the other hand, no significant differences in total serum anti-oxidant levels between the HCC patients with and without periodontitis were observed in this study. This suggests that having periodontitis is not sufficient to induce a decrease in serum anti-oxidant status in HCC

Table 4. Serum level of total bilirubin and albumin in hepatocellular carcinoma patients with and without periodontitis

Parameters	HCC + P (n = 31)	HCC + H (n = 33)	p-value*
Total bilirubin [mg/dl, median (25%, 75%)]	1.3 (0.9, 1.6)	0.8 (0.6, 1.1)	0.002
Albumin [g/dl, median (25%, 75%)]	3.6 (3.2, 4.0)	3.8 (3.4, 4.2)	0.208

*Mann–Whitney U-test.

Table 5. Serum ROM and total anti-oxidant status of hepatocellular carcinoma patients with and without periodontitis and healthy volunteers

Parameters	HCC + P (n = 33)	HCC + H (n = 31)	Control (n = 15)
ROM [CARR U, median (25%, 75%)]	482 (454, 538)*,‡	383 (351, 437)*	302 (290, 322)
OXY [μmol HClO/ml, median (25%, 75%)]	279 (252, 290)*	287 (263, 317)†	311 (306, 323)

*$p < 0.001$, compared with the control.
†$p < 0.01$, compared with the control.
‡$p < 0.001$, compared with the HCC + H group.

patients. Clinical studies have reported that total blood levels of anti-oxidants in subjects with periodontitis were lower than those in subjects with healthy gingiva (Baltacıoğlu et al. 2006, Konopka et al. 2007). However, the decrease in serum anti-oxidant levels following periodontitis may have little effect on the progression of HCC. In addition, several methodologies are now available to evaluate antioxidant status in the blood and a clinical study has demonstrated that enzymatic anti-oxidant activities were significantly higher, whereas levels of non-enzymatic anti-oxidants were significantly lower in periodontitis patients than in control subjects with healthy gingiva (Panjamurthy et al. 2005). Therefore, it is also possible that the relationships among periodontitis, HCC and blood level of anti-oxidants differ depending on the anti-oxidant molecules examined.

Various studies have demonstrated the relationship between periodontitis and cancer. An epidemiological study has reported positive associations between periodontitis and lung cancer mortality above and beyond adjustment for known risk factors for lung cancer (Hujoel et al. 2003). A cross-sectional study has also demonstrated that the degree of alveolar bone loss is associated with an increased risk of tongue cancer (Tezal et al. 2007). Furthermore, a hospital-based case-control study revealed that patients with periodontitis were more likely to have poorly differentiated squamous cell carcinoma in the oral cavity when compared with those without periodontitis (Tezal et al. 2009). These reports are in agreement with the present study that periodontitis may affect HCC.

Epidemiological studies have suggested a positive association between periodontal condition and blood levels of hepatic parameters, such as aspirate aminotransferase (Saito et al. 2006), cholinesterase (Saito et al. 2006) and alanine aminotransferase (Furuta et al. 2010). Animal studies have also indicated that experimental periodontitis is able to induce oxidative damage in the liver and hepatic inflammation with increasing serum levels of ROS (Tomofuji et al. 2007, 2008). Furthermore, a clinical study has demonstrated that periodontal infection is a potential source of infection in the formation of pyogenic liver abscess (Ohyama et al. 2009). These observations and the present results support the notion that maintaining and/or improving periodontal health may offer clinical benefits on hepatic health, even in HCC patients. However, longitudinal clinical studies are necessary to examine the causal relationship between periodontal and hepatic conditions to clarify this issue.

Our study has some limitations. First, it is reported that malnutrition is an independent risk factor of the increased Child-Pugh score (Alberino et al. 2001) and that low blood micronutrient level is associated with periodontitis (Van der Velden et al. 2011), suggesting that nutritional state also has an influence on progression of HCC and periodontitis. The records of nutritional state would thus be necessary to increase the reliability of our data. Next, subject with JIS scores ≤ 2 accounted for more than 70% of the current HCC patents (Table 3). As these patients exhibited mild or moderate stage of HCC, it is possible that the actual relationship between periodontitis and severe stage of HCC differs from the present findings. Third, because this was a cross-sectional study, the causal relationship between periodontitis and HCC still remains unclear. Finally, adjusting socio-economic factors and history of cirrhosis will improve the reliability of the present relationship between periodontitis and stage of HCC.

In conclusion, JIS score and circulating ROS levels in HCC patients with chronic periodontitis were higher than those in periodontally healthy HCC patients. Increased serum levels of ROS following periodontitis may therefore be detrimental to hepatic health in HCC patients.

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Clinical Relevance

Scientific rationale for the study: Systemic increases in ROS induced by periodontitis may affect HCC. However, the relationships among periodontitis, HCC and circulating ROS are unclear.

Principal findings: The JIS score and circulating ROS level in HCC patients with chronic periodontitis were higher than those in HCC patients with periodontally healthy control.

Practical implications: Maintaining and/or improving periodontal health may be beneficial to the hepatic health in HCC patients.