Changes in interleukin-6 levels in saliva in patients with oral squamous cell carcinoma.

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Abstract

Objective: To elucidate the changes in interleukin-6 (IL-6) levels in whole saliva during the treatment phase in patients with oral squamous cell carcinoma (OSCC).

Study Design: Twenty-nine consecutive inpatients with OSCC were enrolled in this study. Stimulated saliva was collected three times (just after hospitalization)[period 1], just before main treatment (surgery: 26/29 cases)[period 2] and at the time of discharge [period 3]. The mean intervals were 11±8 days between periods 1 and 2 and 30±18 days between periods 2 and 3. As controls, 19 age-matched healthy volunteers were also recruited. IL-6 concentrations were measured by a highly sensitive chemiluminescent enzyme immunoassay.

Results: IL-6 was detected in 23/29 samples in the patient group in period 1. The concentration of IL-6 was significantly higher in the patient group (mean: 20.1±36.3 pg/mL) than in the controls (0.6±0.8 pg/mL)(P = 0.003). In the patient group, the mean concentration of IL-6 in period 2 was 43.6±95.6 pg/mL, significantly higher than in period 1 (P=0.002), and 17.1±27.6 pg/mL in period 3 (P=0.52: compared with period 2).

Conclusions: IL-6 was up-regulated in saliva in the OSCC patients. IL-6 level tended to increase before treatment, and returned to baseline levels at discharge.

Introduction

The number of patients with oral cancer is increasing gradually (especially in younger people). [1] Although the diagnostic modalities and therapeutic management of oral cancer is improving, the treatment outcome and prognosis of oral cancer have improved little. [2] The overall 5-year survival rates for oral cancer have remained low at ~30 to 50%. [3] Some researchers pointed out the imperative need for a sensitive biomarker to improve early detection of oral cancer. [1-4] Circulatory tumor markers for oral squamous cell carcinoma (OSCC) were investigated in various studies and showed relatively moderate sensitivity and specificity values with relation to diagnosis, prognosis predicting, or treatment monitoring. [1-6] Saliva has many advantages as a sample over both serum and tissues. [7] Saliva is relatively easy to collect in sufficient quantities for analysis even in the small clinic or the laboratory.

It is recognized that numerous cytokines have various roles in the diseases of the oral mucosa.[8, 9] Interleukin-6 (IL-6) is a multifunctional cytokine that participates in the inflammatory and immune responses.[10-12] Pro-inflammatory cytokines, such as IL-6, have been shown to directly promote the growth of certain types of cancer, and is associated with increased the rate of metastasis.[2, 13] However, IL-6 has dual effects, inhibiting the growth of some cells while stimulating the growth of others. [14] IL-6 is currently being assessed for its ability to promote or inhibit various types of tumors. [2, 4, 9] Some previous studies indicated that the concentrations of IL-6 in serum and saliva are significantly elevated in patients with oral neoplastic and preneoplastic lesions as compared with controls.[8, 9, 15-18] Rhodus et al.[9] reported that OSCC as well as oral preneoplastic lesions had significantly

higher salivary levels of IL-6 than controls. They suggested that the progression of OSCC may be enhanced by the continued expression of the proinflammatory cytokines. St. John et al. [2] examined IL-6 at the messenger RNA (mRNA) and protein levels in both the serum and unstimulated saliva of OSCC patients and age- and gender- matched controls. They demonstrated that IL-6 at both the mRNA and protein levels was detected in higher concentrations in the serum of patients with OSCC compared with controls. However, they did not find significant differences in the salivary concentrations of IL-6 between the two groups at either the mRNA or the protein level. Some researchers suggested that IL-6 in saliva and serum may be a useful biomarker for patient screening. [2, 9, 18] However, the clinical significance of IL-6 in saliva in patients with OSCC is not clearly understood. Since previous studies reported only baseline salivary IL-6 concentrations, [2, 9, 15, 18] it is also uncertain whether IL-6 in saliva would change depending on the various treatment phases. If the concentrations of IL-6 in saliva change during the treatment phase, we have to be concerned about appropriate time to collect saliva samples. Here, however, we do not know when we can collect valuable saliva for early detection or predicting the prognosis of OSCC. We hypothesized that the elevated levels of IL-6 in saliva dropped rapidly after treatment in patients with OSCC. This information may enable the choice of an appropriate time to collect saliva samples in evaluating patient's condition.

This preliminary study was performed to elucidate the changes in IL-6 levels in saliva during the treatment phase in patients with OSCC, and the correlations between IL-6 and clinical factors.

Materials and Methods

Patients

A total of 29 consecutive subjects (11 women and 18 men) with histologically proven OSCC were enrolled in this prospective study (Table 1). All of the subjects were hospitalized for examination and treatment. The patients ranged in age from 32 to 85 years old (mean, 69 years). The duration of diseases ranged from 1 to 12 months (mean: 4.6±3.0 months; median, 4 moths). The primary sites of cancer were; tongue (9 cases), upper gingiva (8 cases), lower gingiva (4 cases), buccal mucosa (4 cases), floor of the mouth (2 cases), and palate (2 cases).

TN-classification was made in accordance with the criteria of the head and neck tumors by WHO (2005). With regard to the T-stage, 3 cases (10%) were classified as T1, 16 cases (55%) as T2, 5 cases (17%) as T3, and 5 cases (17%) as T4a. With regard to N-stage, 24 cases (83%) had no cervical lymph node metastasis (N0), and 2 and 3 cases were classified as N1 (7%) and N2b (10%), respectively.

Histopathological evaluation was performed using a hematoxylin and eosin-stained preparations from the pre-treatment biopsy specimens by two of the authors (YY and JS) who were unaware of which patients the specimens came from. The degree of histological differentiation was determined in accordance with the WHO criteria published in 1997. Twenty cases (70%) had Grade 1 cancer and the remaining 9 cases (30%) had Grade 2 cancer.

The histological mode of invasion was classified according to Yamamoto and Kohama (YK classification system).[19] In this system, cancer classified

as YK-1 has a well-defined borderline. YK-4 has diffuse growth or diffuse invasion. The 29 cases were classified as follows: YK-1, 3 cases (10%); YK-2, 7 cases (24%); YK-3, 8 cases (28%); and YK-4, 11 cases (38%).

With regard to the treatment modality, 26 patients (89%) underwent surgery. Of the remaining 3 patients, 2 were treated with radiation therapy and 1 was treated with chemotherapy. At the time discharge, two patients (numbers 2 and 24) had definite residual cancer in the primary sites.

Nineteen healthy age-matched volunteers (10 women and 9 men) were also recruited as controls. The control subjects ranged in age from 41 to 92 years, with a mean age of 67 years (Table 2). All of the patients and controls were Japanese (single race). There was no significant difference between the patients and control groups in terms of gender (chi-square: 1.0). None of these patients and controls had a history of autoimmune diseases and hepatitis.

Although 12 of the patients were smokers, none of the patients smoked during the period of hospitalization (Table 1). Only five controls smoked. Twenty-five (86%) and thirteen (68%) cases of the patient and the control groups have been taking medicine regularly because of the systemic diseases, such as hypertension, heart disease, diabetes and cerebrovascular disorders.

All of the participants, including the healthy volunteers, gave their informed consent to collection of saliva for the purpose of the study. This study was approved by the Institutional Review Boards of Hokkaido University, Graduate School of Dental Medicine (2006).

Periodontal disease

We divided the patients into two groups according to the presence or absence of periodontal diseases according to periodontal status (CPI) by WHO.[8, 20] In the present study, 10 patients (34%) and 7 controls (37%) were defined as having periodontal disease (Table 1 and 2).

Stimulated saliva sample preparation

All samples of stimulated saliva were collected using a commercially available device (Salivette®; Sarstedt Ltd, Leicester, UK), which has been used for salivary analyses in many previous studies.[21-23] All of the patients and controls were instructed not to eat for one hour prior to sampling. Saliva samples were obtained before lunch, between 10:00 and 12:00, in accordance with the method of Menon.[23] Saliva samples were collected by chewing the Salivette® cotton for 1 min, and depositing the cotton into the collection tube provided. The collection tubes were immediately centrifuged at 2 000 rpm (800 g) for 10 min to obtain whole saliva and stored at –80°C until assay.

Saliva was collected three times in the patient group: just after hospitalization (period 1), just before main treatment (i.e., surgery, radiation and/or chemotherapy)(period 2), and at the time of discharge (period 3). The mean interval between periods 1 and 2 was 11±8 days, while that between periods 2 and 3 was 30±18 days. Definite cancer growth (more than 20% of the diameter from clinical findings or CT findings) was observed in four patients (numbers 2, 10, 24 and 25) between periods 1 and 2. Between periods 1 and 2, the patients received general medical check-

ups, instruction on oral health care, and imaging examination, including computerized tomography (CT), magnetic resonance imaging (MRI), ultrasonic graphy (USG), and/or positron-emission tomography (PET). During this period, one patient (number 28) received adjuvant chemotherapy with oral S-1 (Taiho Pharmaceutical Co., Ltd, Tokyo, Japan), and 11 (numbers 5, 8, 10, 15, 17, 18, 22, 23, 27, 28 and 29) underwent tumor biopsies of cancer. S-1 is an oral anticancer agent comprised of tegafur, 5-chloro-2, 4-dihydroxypyridine, and potassium oxonate. [24] No patients became tumor-free after biopsy or adjuvant chemotherapy. None of the patients received pre-operative radiation. Between periods 2 and 3, four (numbers 1, 2, 15 and 28) and four (numbers 8, 18, 24 and 27) patients received chemotherapy and radiation therapy, respectively. In the control group, saliva was collected once.

The volume of saliva ranged 0.3-2.6 mL (mean: 1.8 mL). Samples less than 0.5 mL in volume or those that contained an excessive amount of blood were excluded from the study. Five samples each obtained during periods 2 and 3 were excluded. In the period 2, three and two samples were excluded because of shortage of volume and mixing of blood, respectively. In the period 3, four and one samples were excluded because of shortage of volume and mixing of blood, respectively. We needed more than 0.5 mL in volume of saliva to measure IL-6 levels in this study. The samples available for assay were from 29 cases in period 1 and 24 cases in each of periods 2 and period 3.

Blood tests for C-reactive protein (CRP) were performed on the patients at the time of hospitalization (period 1). All of these assay were performed in

our hospital laboratory by particle enhanced turbimetric immunoassay with a high sensitivity CRP flex reagent cartridge (normal range: 0-0.3 mg/dL).

Measurement of IL-6 concentrations.

IL-6 concentrations were measured by a highly sensitive chemiluminescent enzyme immunoassay (CL-EIA) in duplicate analyses using mouse monoclonal antibodies against recombinant IL-6 (Fujirebio Inc., Tokyo, Japan).[25] The intra-day and inter-day coefficients of variation (CV) of this assay were 1.5-5.5% and 3.3-4.5%, respectively. This assay format is based on the two-step sandwich CL-EIA method as follows: aliquots 50 μL of each sample were added to 250μ L of suspension of ferrite microparticles coated with a monoclonal antibody against IL-6 as a solid phase and incubated for 10 min at 37°C. Then, the particles were separated magnetically and washed. Aliquots of 250 μL of another monoclonal antibody against IL-6 conjugated with alkaline phosphatase were added to the particles. After 10 min of incubation at 37 °C, the particles were similarly washed. Subsequently, the substrate solution [AMPPD; 3-(2'-spiroadamantane)-4-methoxy-4-(3" phosphoryloxy) phenyl-1, 2-dioxetane disodium] was added at 37 °C. After 5min of incubation, the chemiluminescent signal was photon-counted, using a fully automated CL-EIA analyzer (Lumipulse fFsystem; Fujirebio Inc., Tokyo, Japan). Positive and negative controls consisted of pooled serum samples with three concentrations of IL-6 (low, moderate and high) in the present study. The minimum level of detection of IL-6 in this assay was at 0.3 pg/mL. All samples were assayed at the same time.

Statistical analysis

The Mann-Whitney U test was used to check for differences in IL-6 concentration in saliva samples between the patient and control groups. The Wilcoxon rank sum test was used to compare the IL-6 concentrations of saliva in three periods (periods 1 to 3). Spearman's correlation coefficient was used to assess the correlation between the concentration of IL-6 in saliva and the clinicopathological factors. Stat View J-5.0 statistical software (Abacus Concepts, Berkeley, CA, USA) was used. *P* values less than 0.05 were considered statistically significant.

RESULTS

IL-6 concentrations in saliva samples

We detected IL-6 in 23 of the 29 samples (79%) in the patient group at the time of hospitalization (period 1). The mean±SD (median) concentration of IL-6 in the 29 samples was 20.1±36.3 (3.1) pg/mL and ranged from 0 to 125 pg/mL. In the control group, IL-6 was detected in 11 of the 19 samples and the mean±SD (median) was 0.6±0.8 (0.3), ranging from 0 to 2.3 pg/mL. The concentration of IL-6 in the saliva samples was significantly higher in the patients group than that in the control group ($p = 0.003$) (Figure 1).

Changes in concentrations of IL-6 in saliva samples

Just before main treatment (period 2), we detected IL-6 in 21 of the 24 samples (88%) in the patient group; the mean±SD (median) concentration of IL-6 was 43.6±95.6 (4.5) pg/mL and ranged from 0 to 432 pg/mL. At the time of discharge (period 3), we detected IL-6 in saliva in 22 of the 24 samples (93%); the mean±SD (median) concentration of IL-6 was 17.1±27.6 (5.5) pg/mL and ranged from 0 to 126 pg/mL. The concentration of IL-6 was significantly higher in the sample obtained in period 2 than those from period 1 (p = 0.002). There were no significant differences in the IL-6 concentrations between periods 2 and 3 (p= 0.52) or periods 1 and 3 (p=0.41)(Figure 1).

Clinicopathological data

In the patient group, there were no significant correlations between IL-6 concentrations in the saliva (period 1) and gender (p=0.83), age (p=0.33), duration of disease (p=0.39), T-stage (p=0.80), or clinical stage (p=0.80)(Table 3). Moreover, there were no significant correlations between the IL-6 concentrations and the degree of histological differentiation of cancer (WHO criteria)(p=0.34) or histological mode of invasion (Y-K)(p=0.08).

There were no significant differences in IL-6 concentrations (period 1) between the smokers and non-smokers (p=0.48) or between patients with and without periodontal disease (p=0.37). There was no significant correlation between IL-6 concentration in period 1 and serum CRP (p=0.08). L-6 concentrations were not influenced by the provision of a

biopsy or no biopsy (Table 3). Pre-operative chemotherapy did not influence the salivary IL-6 concentrations significantly (case 28).

Discussion

To our knowledge, there have been no previous studies of the changes in the levels of IL-6 in saliva during the treatment phase in patients with OSCC. In the present study, the concentration of IL-6 in stimulated saliva in patients with OSCC was significantly higher than that in controls, and was not reduced to the levels of controls at discharge (post-treatment days: 30±18 days). These findings may suggest that OSCC patients produce increased release of IL-6 into saliva and that IL-6 contributes to carcinogenesis of oral mucosa and maintenance of condition with OSCC. It is believed that proinflammatory cytokines play an important role in carcinogenesis.[8, 26, 27] and IL-6 inactivates p53 tumor suppressor gene.[26] In contrast, IL-6 may modulate local and systemic immune responses to tumors [15] and may have a direct anti-proliferative effect on tumor cells. [13, 28-30] Although the exact mechanism by which IL-6 influences oral tumor cells is not known, our findings may support the hypothesis that up-regulation of IL-6 during the treatment period shows activation of the local immune system, resulting in inhibition of growth of cancer. However, previous studies indicated another possibility that chronic elevation of IL-6 may also lead to immune unresponsiveness, which is often observed in patients with SCC who have a poor prognosis.[2, 13] St. John et al. [2] demonstrated elevated levels of IL-6 at both the mRNA and protein in the serum in OSCC patients. They mentioned that elevation of IL-6 has been shown to promote immune unresponsiveness and induction of wasting,

cachexia and hypercalcemia all of which are observed in patients with OSCC who have a poor prognosis. Since previous studies reported only baseline salivary IL-6 concentrations, [2, 9, 18] information on changes in salivary IL-6 levels may help determine the time at which patient saliva samples should be collected to evaluate tumor conditions.

Prior to this study, we expected that the levels of IL-6 would decrease rapidly after treatment of cancer. However, the levels of IL-6 at the time of discharge were not decreased significantly. The mean interval between treatment and discharge was 30±18 days. There are five possible explanations for this unexpected phenomenon. One possible explanation may be the growth of cancer. However, 25 of the 29 patients (86%) did not show definite cancer growth during this period. Moreover, at the time of discharge, as only 2 of the 29 patients had definite cancer in the mouth, the remaining 27 (93%) were considered clinically cancer-free. Another possible explanation may be stimulation of inflammation by the biopsy or preoperative chemotherapy, or residual inflammatory reactions after treatment. Although, in the present study, 11 patients received biopsy between periods 1 and 2, IL-6 concentrations were not influenced by the provision of a biopsy or no biopsy. Moreover, IL-6 concentrations were not influenced by pre-operative chemotherapy either. No patients received preoperative radiation in the present study. Moreover, 27 of the 29 patients discharged form the hospital after improving their systemic and local conditions sufficiently. Their body temperatures were normal, and the conditions of the wound at surgical sites were also stable at the time of discharge (data not shown). The third possibility is that the time interval was too short to observe a decrease in IL-6 concentrations. The mean interval between period 2 and 3 was 30±18 days. In previous studies of

patients with oral lichen planus, serum and salivary IL-6 levels were decreased significantly at a mean 42 days and 0.5-3 months (15-90 days) after treatment. [12, 31] We cannot compare these results with ours due to different patient characteristics. However, we cannot deny the possibility that the salivary concentrations of IL-6 might have come down to the levels of controls, if we had collected saliva from the OSCC patients at the time of 2-3 months after treatment. A fourth possibility is that salivary IL-6 concentrations may remain elevated in patients predisposed to tumor recurrences. At this time, however, this cannot be determined. The fifth possibility is that the elevated IL-6 levels after treatment may be due to the enrichment of saliva caused by the dry mouth symptoms associated with cancer treatment. To avoid the influence of saliva enrichment, we excluded saliva samples with volumes of less than 0.5 mL in the present study. However, only this consideration may be insufficient to avoid the influence of enrichment of saliva. However, recent study demonstrated an interesting result. Boras et al. [32] stated that xerostomia as a result of drug intake did not alter IL-6 levels in saliva.

In previous two studies, mean IL-6 concentrations before treatment, as determined by ELISA, in unstimulated saliva from 13 and 19 patients with OSCC, has been reported to be 88.2±43.2 pg/mL and 86.5±84.7 pg/mL, respectively, [17, 18] higher than the concentrations we measured in stimulated saliva during periods 1 and 2. These findings suggest that our saliva samples may have been diluted. On the contrary, stimulation with chewing the cotton might activate the release of IL-6 from the tumor and gingiva. In our patients with OSCC, we think it was pretty difficult to collect enough volume of unstimulated saliva in a short time. As we collected saliva from the patients three times, we would like to ease the burden of the patients.

However, since we collected stimulated saliva three times by the same manner, we think our comparisons are valid.

It is clear that the majority of IL-6 in saliva comes from the local region. Tumor size (T-stage) has been reported to be an independent predictor of survival in patients with OSCC. [14] Large tumors may secrete higher amounts of IL-6. We found, however, no significant correlation between the IL-6 concentrations and T-stage. IL-6 is produced by various cells types, including lymphoid cells, monocytes, fibroblasts, endothelial cells, oral epithelial cells, and several types of tumor cells.[8, 10, 11] Sun et al.[12] suggested that IL-6 was produced by SCC, and contributed to the progression of the disease. We could not measure the serum levels of IL-6 in the patients and controls in the present study. St. John et al. [2] reported the mean concentrations of IL-6 in the serum were 86.5 pg/mL and 0 pg/mL, in patients with OSCC and healthy controls, respectively. Sun et al. [12] reported that the mean serum levels of IL-6 were 3.4±3.1 pg/mL and 2.0±1.5 pg/mL in patients with erosive oral lichen planus and healthy subjects, respectively. From the results of the previous and present studies, it is natural that IL-6 in saliva was produced by not only cancer cells but also peripheral circulation. Moreover, in local environments, the secretion of IL-6 may depend on not only on cancer cells but also on inflammatory reactions.

A variety of factors may influence the physiological characteristics of whole saliva, including time of collection, meals, medication use, periodontal inflammation, smoking or physical activity.[33, 34] In the present study, all of the patients were inpatients. All of the patients and controls were instructed not to eat one hour prior to the sampling.[23] Saliva samples

were obtained before lunch, between 10:00 and 12:00, to reduce the influence of meals, exercise, and circadian rhythms. There were no obvious correlations between the levels of IL-6 and the presence of periodontal disease in the present study. In accordance with a report of Rhodus et al.,[9] we felt it appropriate to exclude subjects taking drugs that induce hyposalivation, such as anticholinergic, antihistamine, and antihypertensives agents. In the present study, however, most patients were elderly, and it was not possible to exclude patients taking drugs associated with hyposalivation, as the most (25 cases) were taking such drugs routinely. Recently, an interesting finding was reported that there was no significant correlation between levels of salivary IL-6 and number of medications taken in patients with drug-induced xerostomia.[32]

In conclusion, the concentration of IL-6 in whole saliva in patients with OSCC was significantly higher than that in controls. The concentration of IL-6 changed during the treatment period, and returned to baseline levels at discharge (post-treatment days: 30±18 days). We intend to continue our research efforts to determine whether the continuous elevation of IL-6 in saliva would influence the prognosis of OSCC.

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References

1. Nagler R, Bahar G, Shpitzer T, Feinmesser R. Concomitant analysis of salivary tumor markers- A new diagnostic tool for oral cancer. Clin Cancer Res 2006;12:3979-84.

2. St. John MAR, Li Y, Zhou X, Denny P, Ho CM, Mintemagno C et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004;103:929-35.

3. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, et al. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res 2008;14:6246-52.

4. Wang YF, Chang SY, Tai SK, Li WY Wang LS. Clinical significance of interleukin-6 and interleukin-6 receptor expressions in oral squamous cell carcinoma. Head Neck 2002: 24:850-8.

5. Nagler RM, Barak M, Ben-Aryeh H, Peled M, Filatov M, Laufer D. Early diagnostic and treatment monitoring role of Cyfra 21-1 and TPS in oral squamous cell carcinoma. Cancer 1999;35:15-25.

6. Kuo WR, Lee KW, Ho KY, Tsai SM, Chiang FY, Juan KH. Tissue polypeptide antigen, carcinoembryonic antigen, carbohydrate antigen, and CA125 levels as tumor markers in squamous cell carcinoma of the head and neck. Kaohsiung J Med Sci 1999;15:152-8.

7. Lawrence HP. Salivary markers of systemic diseases: Noninvasive diagnosis of disease and monitoring of general health. J Can Dent Assoc 2002;68:140-4.

8. Brailo V, Vučićević-Boras V, Cekić-Arambašin A, Alajbeg IŽ, Milenović A, Lukač J. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. Oral Oncol 2006;42:370-3.

9. Rhodus NL, Cheng B, Myers S, Bowles W, Ho W, Ondrey F. A comparison of the pro-inflammatory, NF-kB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. Clin Immunol 2005;114:278–83.

10. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol 1993;54:1–78.

11. Nishimoto N, Kishimoto T, Yoshizaki K. Anti-interleukin 6 receptor antibody treatment in rheumatic disease. Ann Rheum Dis 2000;59 (Suppl 1):i21–7.

12. Sun A, Chia JS, Chang YF, Chiang CP. Serum interleukin-6 level is a useful marker in evaluating therapeutic effects of levamisole and Chinese medicinal herbs on patients with oral lichen planus. J Oral Pathol Med 2002;31:196–203.

13. Smith CW, Chen Z, Dong G, Loukinova E, Pegram MY, Nicholas-Figueroa L, et al. The host environment promotes the development of primary and metastatic squamous cell carcinomas that constitutively express proinflammatory cytokines IL-1α, IL-6, GM-CSF, and KC. Clin. Exp. Metastasis 1998;16:655–64.

14. Khandavilli SD, Ceallaigh Pó, Lloyd CJ., Whitaker R. Serum C-reactive protein as a prognostic indicator in patients with oral squamous cell carcinoma. Oral Oncol 2009:45:912-4.

15. Jablonska E, Piotrowski L, Grabowska Z. Serum levels of IL-1-β, IL-6, TNF-α, TNF-R1 and CRP in patients with oral cavity cancer. Pathol Oncol Res 1997;3:126-9.

16. Nakano Y, Kobayashi W, Sugai S, Kimura H, Yagihashi S. Expression of tumor necrosis factor-α and interleukin 6 in oral squamous cell carcinoma. Jpn J Cancer Res 1999;90:858-66.

17. Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-kappaB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detect Prev 2005;29:42–5.

18. Katakura A, Kamiyama I, Takagi R, Yamada M, Shouno T, Takano N, et al. Comparison of cytokine in saliva from oral cancer patients and normal person. Jpn J Head Neck Cancer (in Japanese) 2006:32:45-50.

19. Yamamoto E, Kohama G, Sunakawa H, Iwai M, Hiratsuka H. Mode of invasion, bleomycin sensitivity, and clinical course in squamous cell carcinoma of the oral cavity. Cancer 1983;51:2175–80.

20. World Health Organization. Oral Health Surveys. Basic Methods. Geneva 1997, World Health Organization.

21. Gröschl M, Rauh M. Influence of commercial collection devices for saliva on the reliability of salivary steroids analysis. Steroids 2006;71:1097–100.

22. Henderson L, Muir M, Mills PR, Spence E, Fox R, McCruden EA, Bagg J. Oral health of patients with hepatic C virus infection: a pilot study. Oral Diseases 2001;7:271–5.

23. Menon R, McIntyre JO, Matrisian LM, Fortunato SJ. Salivary proteinase activity: A potential biomarker for preterm premature rupture of the membrane. Am J Obstet Gynecol 2006;194: 1609

24. Shirasaka T, Shimamoto Y, Fukushima M. Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res 1993;53:4004-9.

25. Shikano M, Sobajima H, Yoshikawa H, Toba T, Kushimoto H, Katsumata H, et al. Usefulness of a Highly Sensitive Urinary and Serum IL-6 Assay in Patients with Diabetic Nephropathy. Nephron 2000;85:81–5.

26. Hodge DR, Peng B, Cherry JC, Hurt EM, Fox SD, Kelley JA, et al. Interleukin 6 supports the maintenance of p53 tumor suppressor gene promoter hypermethylation. Cancer Res 2005;65:4673-82.

27. Philip M, Rowley DA, Schreiber H. Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol 2004;14:433-9.

28. Veltri RW, Rodman SM, Maxim PE, Baseler MW, Sprinkle PM. Immune complexes, serum protein, cell-mediated immunity, and immune regulation in patients with squamous cell carcinoma of the head and neck. Cancer 1986;57:2295–308.

29. Wolf G, Hudson JL, Peterson KA, Miller HL, McClatchey KD. Lymphocyte subpopulations infiltrating squamous carcinomas of the head and neck: correlations with extent of tumor and prognosis. Otolaryngol Head Neck Surg 1986;95:142–52.

30. Karczewska A, Kawrocki S, Bręborowicz D, Filas V, Mackiewicz A. Expression of interleukin-6, interleukin-6 receptor, and glycoprotein 130 correlates with good prognoses for patients with breast carcinoma. Cancer 2000:88:2061-71.

31. Rhoudus Oral Dis: Rhodus NL, Cheng B, Bowles W, Myers S, Miller L, Ondrey F. Proinflammatory cytokine levels in saliva before and agter treatment of (erosive) oral lichen planus with dexamethasone. Oral Dis 2006:12:112-6.

32. Vučićević-Boras V, Brailo V, Lukač J, Kordič D, Picek P, Blažič-Potočki Z. Salivary interleukin-6 and tumor necrosis factor alpha in patients with drug-induced xerostomia. Oral Dis 2006;12:509-11.

33. Chen F, Castranova X, Shi X. New insights into the role of nuclear factor-kappaB in cell growth regulation. Am J Pathol 2001;159:387–97.

34. Khan A, Farah CS, Savage NW, Walsh LJ, Harbow DJ, Sugerman PB. Th1 cytokines in oral lichen planus. J Oral Pathol Med 2003;32:77–83.

Captions to illustration

Figure 1: Salivary IL-6 concentrations in patients and controls, and changes in IL-6 concentration during treatment.

*: Mann-Whitney test, #: Wilcoxon rank sum test. The horizontal and dotted bars show median and mean values, respectively.

Fig. 1

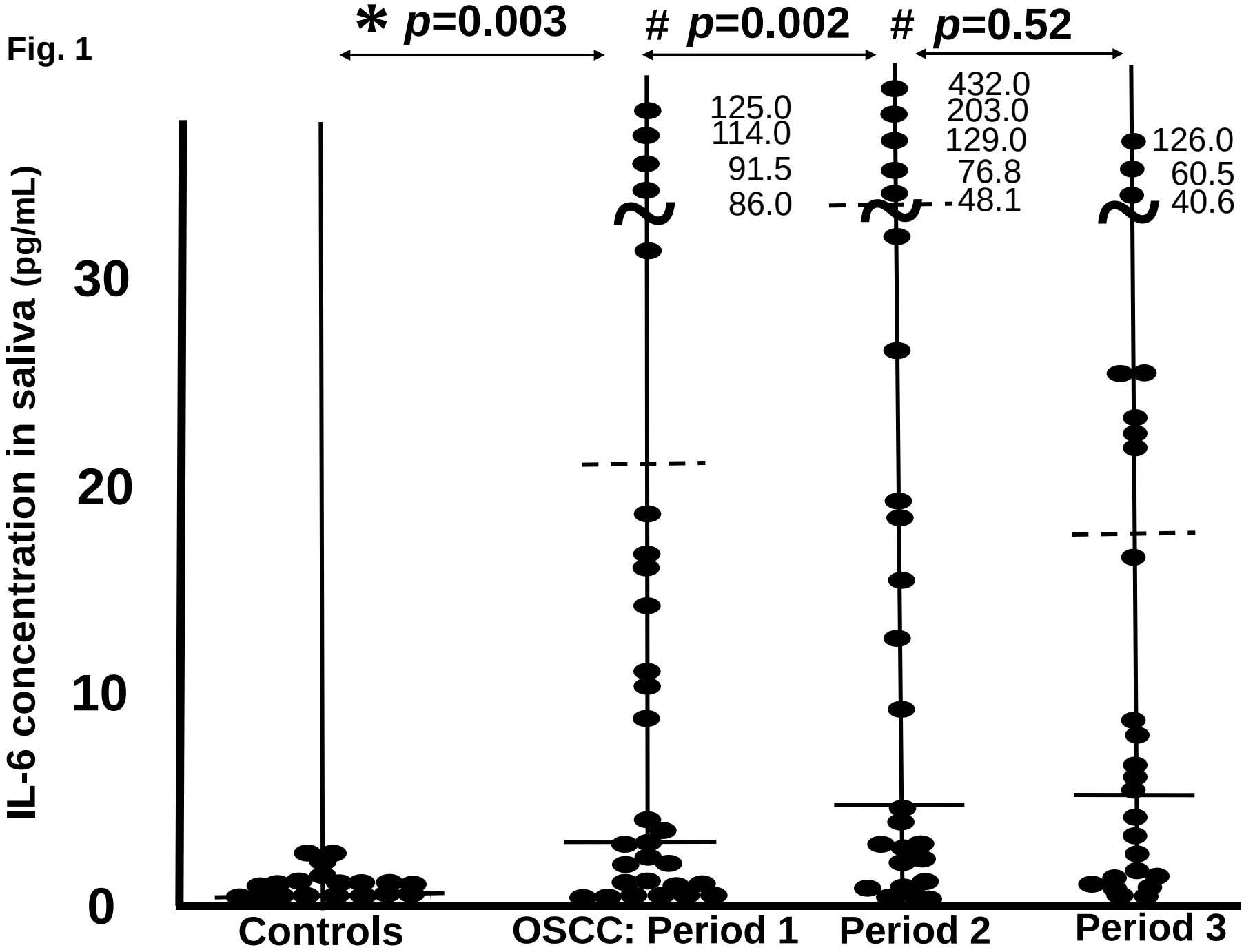
* p=0.003
# p=0.002
# p=0.52
IL-6 concentration in saliva (pg/mL)
0
10
20
30
125.0
114.0
91.5
86.0
432.0
203.0
129.0
76.8
48.1
126.0
60.5
40.6
Controls
OSCC: Period 1
Period 2
Period 3

Table. 1: Patients in this study

Case number	Age / gender (years)	Clinical stage	IL-6 a （pg/mL）	IL-6 b (pg/mL)	IL-6 c (pg/mL)	CRP （μg/dL）	Periodontal disease	Smoker
1	68 / M	I	<0.3	<0.3	<0.3	0.8	Presence	Yes
2	73 / M	IVA	86.0	129.0	7.8	0	Absence	Yes
3	74 / F	III	<0.3	<0.3	-	1.0	Absence	No
4	80 / M	II	<0.3	2.9	2.1	0.7	Presence	Yes
5	59 / M	II	18.7	18.5	16.5	0	Absence	No
6	69 / M	II	4.3	0.4	-	0	Presence	No
7	70 / F	II	<0.3	3.1	60.5	0	Absence	No
8	70 / F	II	<0.3	-	22.4	0	Absence	No
9	70 / M	II	14.7	15.5	40.6	-	Absence	No
10	48 / M	IVA	1.0	9.3	0.5	0.5	Absence	Yes
11	84 / F	II	32.3	-	25.0	0	Presence	Yes
12	72 / M	II	11.0	12.5	8.4	0.5	Absence	Yes
13	85 / M	IVA	8.8	-	23.8	0	Presence	No
14	73 / M	II	0.4	<0.3	<0.3	1.5	Absence	No
15	61 / M	IVA	3.1	4.2	5.2	0.3	Absence	Yes
16	78 / F	IVA	125.0	432.0	0.6	0.3	Absence	No
17	78 / M	II	10.5	48.1	1.7	0	Absence	No
18	32 / F	III	4.1	4.8	5.7	0	Presence	No
19	80 / F	II	114.0	1.7	1.0	0	Absence	No
20	77 / F	III	16.9	25.8	126.0	0	Absence	No
21	70 / F	III	91.5	203.0	-	-	Presence	No
22	63 / M	II	2.2	19.2	4.0	-	Absence	Yes
23	67 / M	II	1.8	1.8	6.9	2.4	Presence	Yes
24	66 / M	III	2.8	76.8	3.6	0	Absence	No
25	70 / F	II	0.3	-	1.0	1.1	Presence	No
26	59 / M	II	16.5	33.6	25.0	-	Absence	Yes
27	62 / M	III	0.6	1.0	1.4	0.9	Absence	Yes
28	61 / F	IVA	<0.3	3.6	21.9	0	Presence	Yes
29	83 / M	II	1.0	<0.3	-	0	Absence	No

IL-6 a: IL-6 concentration in saliva in period 1

IL-6 b: IL-6 concentration in saliva in period 2

IL-6 c: IL-6 concentration in saliva in period 3

CRP: serum CRP level L-6 in period 1

Table. 2: Controls in this study

Case number	Age / gender (years)	IL-6 （pg/mL）	Periodontal disease	Smoker
1	76 / F	1.9	Presence	No
2	68 / M	1.5	Absence	No
3	77 / F	0.5	Absence	No
4	73 / M	0.3	Presence	Yes
5	54 / M	0.3	Presence	Yes
6	92 / F	<0.3	Absence	No
7	55 / F	<0.3	Absence	No
8	41 / M	1.4	Absence	No
9	67 / F	<0.3	Presence	No
10	70 / F	0.5	Absence	No
11	41 / F	<0.3	Absence	No
12	80 / F	2.3	Absence	No
13	70 / M	2.3	Presence	Yes
14	78 / F	0.8	Absence	No
15	80 / M	<0.3	Absence	No
16	57 / F	0.3	Absence	Yes
17	90 / F	<0.3	Absence	No
18	57 / M	<0.3	Presence	No
19	42 / M	<0.3	Presence	Yes

IL-6: IL-6 concentration in saliva

Table 3: Correlations between the IL-6 in saliva and T-stage, duration of disease or biopsy

	IL-6: period 1 Mean ±S.D. (Median)		IL-6: period 2 Mean ±S.D. (Median)		p value
T-stage					
T1/T2: n=19	13.6 ±28.4 (4.1) pg/mL	*	38.0 ±109.5 (15.5) pg/mL	**	* *p*=0.80
T3/T4: n=10	31.0 ±46.3 (3.0) pg/mL		47.7 ±69.5 (6.8) pg/mL		** *p*=0.49
Duration of disease (month)					
1-4: n=16	30.9 ±45.2 (6.6) pg/mL	***			*** *p*=0.39
5-12: n=13	5.6 ±6.6 (3.1) pg/mL				
Biopsy between period 1 and 2					
Yes: n=11	3.9 ±5.7 (1.8) pg/mL	†	11.0±14.7 (4.5) pg/mL	‡	† *p*=0.19
No: n=18	29.1 ±42.8 (9.9) pg/mL		62.4±117.8 (12.5) pg/mL		‡ *p*=0.68