치의학석사 학위논문

Association between Periodontitis and Interleukin-8 in Saliva

치주병과 타액내 Interleukin-8 과의 연관성

2016년 2월

서울대학교 치의학대학원

치의학과

김 현 태

Association between Periodontitis and Interleukin-8 in Saliva

지도교수 김 현 덕

이 논문을 치의학석사 학위논문으로 제출함

2015년 10월

서울대학교 치의학대학원

치의학과

김 현 태

김현태의 치의학석사 학위논문을 인준함

2015년 11월

위 원 장 김 현 만 (인)

부위원장 김 현 덕 (인)

위 원 류 인 철 (인)

Abstract

Association between Periodontitis and Interleukin-8 in Saliva

Hyuntae Kim
Dentistry major
School of Dentistry
Seoul National University

Objective: Periodontitis has been known as chronic inflammatory disease initiated by an unbalance between pathologic oral microbiota and the host defense. Several studies suggested that interleukin-8 (IL-8) is associated with periodontitis in gingival crevicular fluids (GCF) and serum, but the relationship between IL-8 and periodontitis in whole saliva has not been widely reported. This study aims to evaluate the association between periodontal severity and the whole salivary IL-8 level.

Methods: Participants were recruited from the Yangpyeong health cohort study from 2010 to 2014 as the baseline for dental cohort. The final 623 participants were selected for the analysis. Periodontitis was assessed by a dentist using Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP)

criteria through panoramic radiographs. IL-8 was assayed using multiplexed bead immunoassay (Luminex). Information about age, sex, smoking, drinking, and education were obtained by interview. Analysis of variance (ANOVA) analysis was applied to assess crude association between salivary IL-8 and periodontitis, and analysis of covariance (ANCOVA) controlled for various confounders.

Results: Mean salivary level of IL-8 was not discriminated between periodontitis severity groups (ANOVA, p-value=0.235). After controlling for confounders, statistical difference of salivary IL-8 was not observed between groups (ANCOVA, p-value=0.375). Neither age nor sex stratified analyses showed significant difference.

Conclusion: There was no statistically significant difference of salivary IL-8 between periodontitis groups. Further study is indicated to confirm the result.

Keywords: periodontitis, interleukin-8, saliva, molecular epidemiology

Student number: 2012-22157

Index

Tables and Figure Index

Appendix

I. Introduction

Periodontitis is a chronic inflammatory disease initiated by presence of pathologic oral microbiota and impaired host immune defense (Armitage, 2003, Listgarten, 1986). Pathogenesis of periodontitis is a consequence of complex risk factors such as bacteria (Socransky et al., 1998), host immunologic reaction (Kornman, 2008, Sorsa et al., 2004, Kim et al., 2014), genetic factors (Hong et al., 2015), systemic burden (Kim et al., 2010, Han et al., 2012a), and environmental factors (Haber et al., 1993, Preber and Bergström, 1990). In affected periodontium, cascade of destruction (inflammation, connective tissue degradation, and alveolar bone destruction) is observed in clinical settings (Sexton et al., 2011). Early detection of periodontitis is important because the disease leads to irreversible destruction of periodontal tissues and tissue-supporting alveolar bone levels, which causes tooth mobility, tooth loss, and mastication problems (Kim et al., 2014, Kinane, 2001).

Traditional clinical diagnosis of periodontal disease includes the evaluation of clinical parameters and oral radiographs such as pocket depth(PD), clinical attachment loss(CAL), bleeding on probing(BOP), and alveolar bone loss(ABL) (Armitage, 2004). However, these factors fail to give information about the precise site of active disease, high risk group for periodontitis, and susceptibility to future disease progression. Therefore, diagnostic methods in clinical practice today lack the tool to detect risks of periodontitis in advance of onset (Zhang et al., 2009, Giannobile et al., 2009).

Interleukin-8 (IL-8) is a chemokine which is mainly produced by macrophages and epithelial cells when the inflammation begins. It activates and attracts neutrophils to the site of inflammation, and plays an important role in progression of periodontitis. (Harada et al., 1994, Okada and Murakami, 1998). IL-8 is released from the cells in the inflamed periodontium, and it can be detected in saliva.

Several publications have been recently addressed the potential diagnostic properties of saliva. Saliva contains locally produced proteins from gingival crevicular fluid and saliva glands, as well as those from systemic circulation. Therefore, qualitative and quantitative change in the composition of saliva would have a diagnostic and therapeutic significance for periodontitis. (Frodge et al., 2008, Miller et al., 2006). In addition to this, saliva is readily collectable by a non-invasive method, without trained dental professionals (Giannobile et al., 2009, Kaufman and Lamster, 2000).

Several studies have investigated the association between IL-8 and periodontitis in gingival crevicular fluids. However, only two researches showing no association were reported about the role of IL-8 in saliva (Sexton et al., 2011, Teles et al., 2009). Their results were obtained from small numbers of participants without adjusting for confounders. Hence, the research hypothesis is that there is difference in salivary IL-8 level across periodontitis severity. The aim of this study is to evaluate the association between periodontal severity and the whole salivary IL-8 levels.

II. Material and Methods

1. Ethical considerations

This cross-sectional study was reviewed and approved by the Institutional Review Board for Human Subjects of the Seoul National University Dental Hospital (IRB approval number: ERI14001). All participants provided a written informed consent.

2. Study populations

Participants were recruited from the Yangpyeong health cohort study from 2010 to 2014 as the baseline for dental cohort. Dentists carried out complete oral examinations for all patients in a dental unit. The exam involved periodontal probing, oral hygiene evaluation, dental examination, and mucosal evaluation. General health status was evaluated by physicians during the survey. Information about sociodemographic status and general/oral health-related behaviors was obtained from questionnaires through interview. Exclusion criteria were: 1) participants with any missing variable, 2) participants with less than six natural teeth, 3) participants who refused to collect saliva samples. The final 623 participants were selected for the analysis.

3. Assessment of periodontitis

Assessment of periodontitis followed the Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP) criteria (Eke et al.,

2012). Panoramic radiographs were taken by a digital panoramic tomography machine (Pax-Primo, Vatech Global, Seoul Korea). The radiographic alveolar bone loss (RABL) was defined as the vertical distance between cemento-enamel junction (CEJ) and deepest point of alveolar bony pocket. RABL was measured on the mesial and distal side of all teeth by trained dentists. When CEJ was not clearly visible for technical reasons (overlapping teeth or prosthesis), arbitrary CEJ was applied by referring to the CEJ of the adjacent teeth. According to the CDC/AAP criteria, three scales were used to classify severity of periodontitis: normal (including mild periodontitis: two or more interproximal sites with 3mm≤RABL<4mm [not on same tooth]), moderate periodontitis (two or more interproximal sites with 4mm≤RABL<6mm [not on same tooth]) and severe periodontitis (two or more interproximal sites with RABL≥6mm [not on same tooth]).

4. Collection of whole saliva

The non-stimulated whole saliva samples were collected from each participant into sterile conical tubes for 10 minutes (Navazesh, 1993). The participants were instructed to abstain from swallowing during saliva collection. Unstimulated saliva flow rate was measured and analyzed in milliliters per minute. Collected saliva samples were stored into deep freezer at –80℃ until use.

5. Measurement of IL-8 in saliva

Salivary concentration of IL-8 was determined by multiplexed bead immunoassay (Luminex). Human interleukin kits were used as stated in the manufacturers' instructions using 150μL of unstimulated saliva per test. A standard curve was created, and concentration of IL-8 was measured according to the standard curve.

6. Assessment of confounders

Several risk factors of periodontitis were considered in this paper to adjust confounding effects. Face-to-face interviews with structured questionnaires were conducted to evaluate potential confounders. Selected covariates included health-related behaviors (smoking and drinking), a systemic factor (metabolic syndrome), sociodemographic factors (age, sex, and education level) (Kim et al., 2014, Boillot et al., 2011) (Table 1). The questionnaire about smoking was “Have you ever smoke in lifetime? (yes/no)”, Drinking was obtained with the question “Are you a never-drinker? (yes/no)”. Education was obtained using a five scale questionnaire depending on the highest level (1= middle school, 2= high school, 3= college school, 4= graduate school, and 5= the rest), and categorized into two (1=less than middle school, 2=longer than middle school). Metabolic syndrome is a complex collection of symptoms defined as the presence of three or more of five metabolic syndrome components: hypertension, hypertriglyceridaemia and low high-density lipoprotein(HDL) cholesterol but also obesity and insulin resistance (Alberti et al., 2006).

Association of metabolic syndrome and periodontitis was documented in Korean population, so metabolic syndrome was included as a confounder for periodontitis (Han et al., 2012b). Presence of metabolic syndrome followed the International Diabetes' Federation's criteria. Information about sociodemographic and behavioral factors was obtained from structured questionnaires by trained interviewers.

7. Statistical analysis

As continuous variables (age, natural teeth, and flow rate of unstimulated saliva) were not normally distributed (Kolmogorov-Smirnov[K-S], p-value<0.05), characteristics of participants were presented as medians with 1st and 3rd quartiles. Kruskal-Wallis one-way analysis was applied for continuous variables to compare the distribution of variables across periodontitis severity. Categorical variables (sex, smoking, drinking, metabolic syndrome, and education) were presented as frequencies and the prevalence. Chi-square test was applied for categorical variables to test the difference between periodontitis severity.

Since IL-8 was not normally distributed (K-S, p-value<0.001), common logarithmic transformation of IL-8 was applied, and the $\log_{10}$(1+IL-8) was normally distributed (K-S, p-value=0.358) (Fig. 1). Crude association of the levels of IL-8 with the severity of periodontitis was tested using analysis of variance (ANOVA). Adjusted association was tested using analysis of covariance (ANCOVA) controlling for age, sex, number of natural teeth, flow rate of unstimulated saliva, smoking,

drinking, metabolic syndrome, and education. For statistical significance, Type I error was set at p-value of 0.05. Statistical analysis was performed using a computer software program. (SPSS v.22, IBM, Chicago, IL).

III. Results

1. Characteristics of subjects

Those who had periodontitis had fewer natural teeth than normal group, and were more smokers (Table 1). The periodontitis groups reported 10.7% more prevalence of smoking for moderate periodontitis group and 15.2% more prevalence for severe periodontitis group than healthy counterpart. However, differences in age, flow rate of unstimulated saliva, drinking, metabolic syndrome, and level of education were not observed across periodontitis severity.

2. Crude association between salivary IL-8 and periodontitis

In bivariate analyses, the unadjusted mean value of salivary IL-8 was found to be higher in normal group (Table 2). The unadjusted mean±standard deviation concentrations of salivary IL-8 were 2.605±0.702 pg/mL for normal group, 2.539±0.636 pg/mL for moderate periodontitis group, and 2.484±0.607 pg/mL for severe periodontitis group. However, the result did not show a statistically significant difference between groups (ANOVA, p-value=0.235).

3. Adjusted association between salivary IL-8 and periodontitis

In multivariate analyses, the adjusted mean value of salivary IL-8 was found to be higher in normal group, after controlling for confounders such as age, sex, natural teeth, flow rate of unstimulated saliva, smoking, drinking, metabolic syndrome, and education (Table 2). The adjusted mean±standard error concentrations of salivary IL-8 were 2.594±0.042 pg/mL for normal group, 2.545±0.039 pg/mL for moderate periodontitis group, and 2.492±0.060 pg/mL for severe periodontitis group. However, the difference between groups was not significant (ANCOVA, p-value=0.375).

4. Stratified comparison of adjusted salivary IL-8 across periodontitis severity

In sex-stratified analysis, the adjusted level of IL-8 with moderate periodontitis was higher than others in male subgroup, however, which was not statistically significant (ANCOVA, p-value=0.765) (Table 3). In female, normal group showed higher IL-8 level than other periodontitis groups. However, the difference was not significant between periodontitis severity groups (ANCOVA, p-value=0.224). In age-stratified analysis, normal groups exhibited higher salivary IL-8 than other periodontitis groups in both <60 and ≥60 subgroups. However, statistically significant difference was not observed between periodontitis severity and salivary IL-8 level. (ANCOVA, p-value=0.873 for <60 years, p-value=0.287 for ≥60 years).

IV. Discussion

Some studies have investigated that salivary elements can provide meaningful diagnostic information (Kaufman and Lamster, 2000, Giannobile et al., 2009, Zhang et al., 2009). Despite IL-8 is a major mediator in inflammation acting as a chemoattractant for neutrophils, only two studies have investigated the role of IL-8 in the whole saliva (Sexton et al., 2011, Teles et al., 2009). In one study, 6-month case-controlled study was performed, with 33 participants receiving oral hygiene instructions (OHI) alone and 35 with scaling and root planning combined with OHI (Sexton et al., 2011). The level of IL-8 was evaluated longitudinally using multiplex immunosorbent assay, and it did not significantly vary from baseline at 16 or 28 weeks. The other study was the cross-sectional study with 74 subjects having chronic periodontitis and 44 periodontally healthy individuals (Teles et al., 2009). Authors examined the association of interleukin-1β, -2, -4, -5, -6, -8, -10, interferon-γ, and tumor necrosis factor-α with periodontitis in whole saliva using a multiplexed bead immunoassay (Luminex). They showed that salivary concentration of IL-8 was not significantly different between periodontitis group and periodontally healthy group. However, their results were obtained from small number of participants without adjusting for confounders. Hence, this research about association between salivary IL-8 and periodontitis severity was performed. Moreover, this is the first study assessing whether IL-8 in whole saliva is associated with periodontitis severity in Korean population.

The major strength of study is threefold. (1) the sample was large enough to allow us to evaluate relationships, (2) the association was controlled for various confounders, including socio-demographic and behavioral factors, (3) age and sex stratified analysis was conducted according to various confounders.

The present results of the study did not demonstrate significant salivary interleukin-8 difference between periodontitis severity. The result was in agreement with few recent studies in the whole saliva, where salivary inteleukin-8 was not significantly different between periodontitis groups (Sexton et al., 2011, Teles et al., 2009). However, it was in contrast to other reports that have described the positive association between interleukin-8 level and periodontitis severity in gingival crevicular fluids (Tsai et al., 1995, Gamonal et al., 2000). Thus, further study is indicated to clarify the discrepancy.

Part of the rationale using saliva for periodontitis detection is based on the concept that inflammatory mediators find their way from inflamed tissue to oral fluid through gingival crevicular fluid. As levels of cytokines in gingival crevicular fluids are elevated in chronic periodontitis sites compared to periodontally healthy sites, it is speculated that they would also increase in the whole saliva. However, the concentration of cytokines in whole saliva was significantly lowered compared to that in gingival crevicular fluids as the cytokines released from gingival crevicular fluids were diluted in whole saliva. In the present study, the mean concentration of IL-8 in saliva was 1.4 ng/ml. The salivary level of IL-8 was found to be considerably low

compared to that of GCF which was 203 ng/mL (Tsai et al., 1995). The result supported the dilution effect of the gingival crevicular fluid containing cytokines in whole saliva.

Another possible mechanism of lower concentration of salivary IL-8 was the inhibitory effect of whole and parotid saliva. The inhibitory effect of whole and parotid saliva on the levels of cytokines were investigated (Wozniak et al., 2002, Ng et al., 2007). They suggested that salivary IL-8 could be decreased due to the inhibitory effect of mucin-like proteins or other large molecules and enzymatic degradation (Wozniak et al., 2002).

This study has some limitations. First, study was based on the cross-sectional design that did not allow inferring the causative effect of the variables effectively. For future investigations, one might consider a longitudinal study that would explain the cause and effect relationships between periodontitis and IL-8. Second, in this study, samples were analyzed by multiplexed bead immunoassay, which used less sample volume than enzyme-linked immunosorbent assay (ELISA). As interpretation and reporting of cytokine values at low or high concentrations can be especially problematic in multiplexed bead immunoassay (Leng et al., 2008), one might take consideration of using ELISA for confirming the result. Despite of these limitations, our data was sufficient to test the hypothesis that there is difference in salivary IL-8 level across periodontitis severity.

V. Conclusion

Overall, the significant association was not observed between the whole salivary IL-8 level and periodontitis severity. Additional studies must be conducted to confirm this result.

References

ALBERTI, K. G. M. M., ZIMMET, P. & SHAW, J. 2006. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic medicine : a journal of the British Diabetic Association,* 23**,** 469-480.

ARMITAGE, G. C. 2003. Diagnosis of periodontal diseases. *Journal of periodontology*.

ARMITAGE, G. C. 2004. The complete periodontal examination. *Periodontology 2000,* 34**,** 22-33.

BOILLOT, A., EL HALABI, B., BATTY, G. D., RANG , H., CZERNICHOW, S. & BOUCHARD, P. 2011. Education as a Predictor of Chronic Periodontitis: A Systematic Review with Meta-Analysis Population-Based Studies. *PloS one,* 6**,** e21508-9.

EKE, P. I., PAGE, R. C., WEI, L., THORNTON-EVANS, G. & GENCO, R. J. 2012. Update of the case definitions for population-based surveillance of periodontitis. *Journal of periodontology,* 83**,** 1449-1454.

FRODGE, B. D., EBERSOLE, J. L., KRYSCIO, R. J., THOMAS, M. V. & MILLER, C. S. 2008. Bone remodeling biomarkers of periodontal disease in saliva. *Journal of periodontology,* 79**,** 1913-1919.

GAMONAL, J., ACEVEDO, A., BASCONES, A., JORGE, O. & SILVA, A. 2000. Levels of Interleukin-1β, -8, and -10 and RANTES in Gingival Crevicular Fluid and Cell Populations in Adult Periodontitis Patients and the Effect of Periodontal Treatment. *Journal of periodontology,* 71**,** 1535-1545.

GIANNOBILE, W. V., BEIKLER, T., KINNEY, J. S., RAMSEIER, C. A., MORELLI, T. & WONG, D. T. 2009. Saliva as a diagnostic tool for

periodontal disease: current state and future directions. *Periodontology 2000,* 50**,** 52-64.

HABER, J., WATTLES, J., CROWLEY, M., MANDELL, R., JOSHIPURA, K. & KENT, R. L. 1993. Evidence for cigarette smoking as a major risk factor for periodontitis. *Journal of periodontology,* 64**,** 16-23.

HAN, D.-H., LIM, S., PAEK, D. & KIM, H.-D. 2012a. Periodontitis could be related factors on metabolic syndrome among Koreans: a case-control study. *Journal of clinical periodontology,* 39**,** 30-37.

HAN, D.-H., SHIN, H.-S., PAEK, D. & KIM, H.-D. 2012b. Gingival crevicular fluid levels of matrix metalloproteinases cross-sectionally related to periodontitis and metabolic syndrome in community Koreans. *Journal of Clinical Periodontology,* 39**,** 1125-1131.

HARADA, A., SEKIDO, N., AKAHOSHI, T., WADA, T., MUKAIDA, N. & MATSUSHIMA, K. 1994. Essential involvement of interleukin-8 (IL-8) in acute inflammation. *Journal of leukocyte biology,* 56**,** 559-564.

HONG, K.-W., SHIN, M.-S., AHN, Y.-B., LEE, H.-J. & KIM, H.-D. 2015. Genomewide association study on chronic periodontitis in Korean population: results from the Yangpyeong health cohort. *Journal of clinical periodontology,* 42**,** 703-710.

KAUFMAN, E. & LAMSTER, I. B. 2000. Analysis of saliva for periodontal diagnosis. *Journal of Clinical Periodontology,* 27**,** 453-465.

KIM, H.-D., SIM, S.-J., MOON, J.-Y., HONG, Y.-C. & HAN, D.-H. 2010. Association between periodontitis and hemorrhagic stroke among Koreans: a case-control study. *Journal of periodontology,* 81**,** 658-665.

KIM, H.-D., SUKHBAATAR, M., SHIN, M., AHN, Y.-B. & YOO, W.-S. 2014. Validation of periodontitis screening model using sociodemographic,

systemic, and molecular information in a Korean population. *Journal of periodontology,* 85**,** 1676-1683.

KINANE, D. F. 2001. Causation and pathogenesis of periodontal disease. *Periodontology 2000,* 25**,** 8-20.

KORNMAN, K. S. 2008. Mapping the Pathogenesis of Periodontitis: A New Look. *Journal of periodontology,* 79**,** 1560-1568.

LENG, S. X., MCELHANEY, J. E., WALSTON, J. D., XIE, D., FEDARKO, N. S. & KUCHEL, G. A. 2008. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. *The journals of gerontology. Series A, Biological sciences and medical sciences,* 63**,** 879-884.

LISTGARTEN, M. A. 1986. Pathogenesis of periodontitis. *Journal of clinical periodontology*.

MILLER, C. S., KING, C. P., JR., LANGUB, M. C., KRYSCIO, R. J. & THOMAS, M. V. 2006. Salivary biomarkers of existing periodontal disease: a cross-sectional study. *J Am Dent Assoc,* 137**,** 322-9.

NAVAZESH, M. 1993. Methods for collecting saliva. *Annals of the New York Academy of Sciences,* 694**,** 72-77.

NG, P. Y. B., DONLEY, M., HAUSMANN, E., HUTSON, A. D., ROSSOMANDO, E. F. & SCANNAPIECO, F. A. 2007. Candidate salivary biomarkers associated with alveolar bone loss: cross-sectional and in vitro studies. *FEMS Immunology & Medical Microbiology,* 49**,** 252-260.

OKADA, H. & MURAKAMI, S. 1998. Cytokine expression in periodontal health and disease. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists,* 9**,** 248-266.

PREBER, H. & BERGSTR M, J. 1990. Effect of cigarette smoking on periodontal healing following surgical therapy. *Journal of clinical periodontology,* 17**,** 324-328.

SEXTON, W. M., LIN, Y., KRYSCIO, R. J., DAWSON, D. R., EBERSOLE, J. L. & MILLER, C. S. 2011. Salivary biomarkers of periodontal disease in response to treatment. *Journal of Clinical Periodontology,* 38**,** 434-441.

SOCRANSKY, S. S., HAFFAJEE, A. D., CUGINI, M. A., SMITH, C. & KENT, R. L. 1998. Microbial complexes in subgingival plaque. *Journal of clinical periodontology,* 25**,** 134-144.

SORSA, T., TJ DERHANE, L. & SALO, T. 2004. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Diseases,* 10**,** 311-318.

TELES, R. P., LIKHARI, V., SOCRANSKY, S. S. & HAFFAJEE, A. D. 2009. Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. *Journal of Periodontal Research,* 44**,** 411-417.

TSAI, C. C., HO, Y. P. & CHEN, C. C. 1995. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult periodontitis. *Journal of periodontology,* 66**,** 852-859.

WOZNIAK, K. L., ARRIBAS, A., LEIGH, J. E. & FIDEL, P. L. 2002. Inhibitory effects of whole and parotid saliva on immunomodulators. *Oral Microbiology and Immunology,* 17**,** 100-107.

ZHANG, L., HENSON, B. S., CAMARGO, P. M. & WONG, D. T. 2009. The clinical value of salivary biomarkers for periodontal disease. *Periodontology 2000,* 51**,** 25-37.

Table 1. Characteristics of variables by periodontitis severity (N=623)

Variable	Periodontitis severity†			P value*
	Normal (n=239)	Moderate (n=267)	Severe (n=117)	
Age in years, median (25,75)	61(53,68)	62(55,70)	64(56,69)	0.051
Sex, n (%)				**<0.001**
Male	**63(26.4)**	**117(43.8)**	**59(50.4)**	
Female	**176(73.6)**	**150(56.2)**	**58(49.6)**	
Natural teeth, median(25,75)	**25(20,28)**	**24(19,27)**	**22(16,25.5)**	**<0.001**
Flow rate of unstimulated saliva, median(25,75)	0.12(0.06,0.18)	0.13(0.07,0.20)	0.12(0.07,0.20)	0.144
Smoking, n(%)				**<0.001**
No (n=447)	**189(95.0)**	**183(84.3)**	**75(79.8)**	
Yes (n=63)	**10(5.0)**	**34(15.7)**	**19(20.2)**	
No response (n=113)				
Drinking, n(%)				0.417
No (n=281)	115(48.1)	118(44.2)	48(41.0)	
Yes (n=342)	124(51.9)	149(55.8)	69(59.0)	
Metabolic syndrome‡, n(%)				0.144
No (n=405)	144(60.3)	182(68.2)	79(67.5)	
Yes (n=218)	95(39.7)	85(31.8)	38(32.5)	
Level of education, n(%)				0.093
<Middle school (n=556)	210(87.9)	235(88.0)	111(94.9)	
>Middle school (n=67)	29(12.1)	32(12.0)	6(5.1)	

Bold denotes statistical significance at P<0.05.

* P-value obtained from chi-square test for categorical variables, and Kruskal-Wallis test for age, natural teeth, flow rate of unstimulated saliva.

† Periodontitis severity was defined by Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP) criteria. Normal: periodontally healthy and mild periodontitis (two or more interproximal sites with 3mm≤RABL<4mm [not on same tooth]), Moderate periodontitis: two or more interproximal sites with 4mm≤RABL<6mm (not on same tooth), Severe periodontitis: two or more interproximal sites with RABL≥6mm (not on same tooth).

‡ Metabolic syndrome follows the International Diabetes Federation (IDF)'s criteria.

Table 2. Comparison of salivary interleukin-8 (pg/ml) across periodontitis severity.

Salivary IL-8†	Periodontitis severity			P value*
	Normal (n=239)	Moderate (n=267)	Severe (n=117)	
Unadjusted, mean±SD				
$\log_{10}$[1+IL-8]	2.605±0.702	2.539±0.636	2.484±0.607	0.235
(IL-8)	(401.72±5.04)	(345.94±4.33)	(304.79±4.05)	
Adjusted, mean±SE				
$\log_{10}$[1+IL-8]	2.594±0.042	2.545±0.039	2.492±0.060	0.375
(IL-8)	(391.65±1.10)	(349.75±1.09)	(309.46±1.15)	

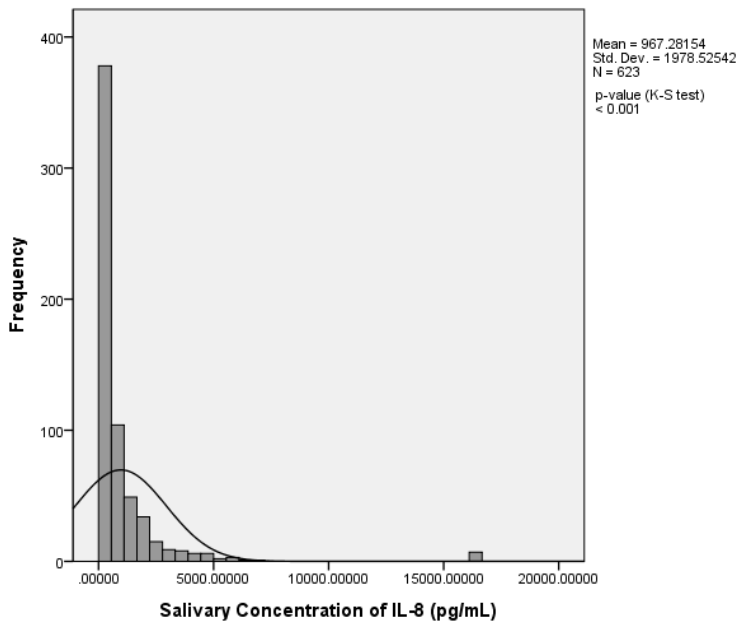
* Data and p value are obtained from analysis of variance (ANOVA) for unadjusted IL-8, and from analysis of covariance(ANCOVA) for adjusted IL-8. Adjusted mean IL-8 are controlled for age, sex, natural teeth, flow rate of unstimulated saliva, smoking(missing=mean value), drinking, metabolic syndrome, and education.
† SD: standard deviation, SE: standard error

Table 3. Stratified comparisons of adjusted salivary interleukin-8 (pg/ml) (mean±standard error) with periodontitis severity according to subgroups

Subgroups category		Periodontitis severity			P value*
		Normal (n=239)	Moderate (n=267)	Severe (n=117)	
Sex					
Male (n=239)	$\log_{10}$[1+IL-8]	2.450±0.077	2.512±0.056	2.461±0.080	0.765
	(IL-8)	(280.84±0.19)	(324.09±0.14)	(288.07±0.20)	
Female (n=384)	$\log_{10}$[1+IL-8]	2.659±0.050	2.561±0.054	2.507±0.087	0.224
	(IL-8)	(455.04±0.12)	(363.92±0.132)	(320.37±0.22)	
Age					
<60 years (n=259)	$\log_{10}$[1+IL-8]	2.536±0.061	2.494±0.060	2.490±0.101	0.873
	(IL-8)	(342.56±0.15)	(310.90±0.15)	(308.03±0.26)	
≥60 years (n=364)	$\log_{10}$[1+IL-8]	2.644±0.058	2.581±0.053	2.492±0.076	0.287
	(IL-8)	(439.55±0.14)	(380.07±0.13)	(309.46±0.19)	

* Data and p value are obtained from analysis of covariance(ANCOVA) adjusted for age, sex, natural teeth, flow rate of unstimulated saliva, smoking(missing-=mean value), drinking, metabolic syndrome, and education except for subgroup.

A.



B.

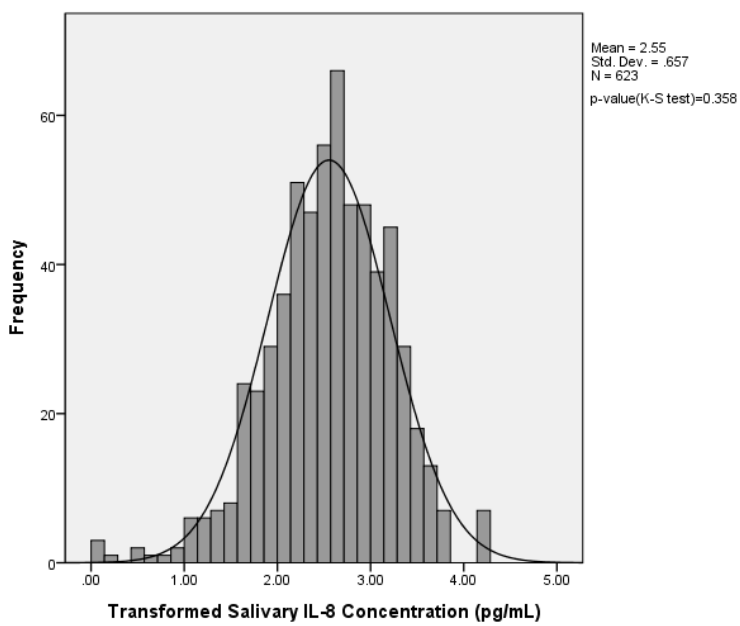


Figure 1. Distribution of salivary IL-8: A. distribution of salivary IL-8 (raw value), Kolo molov-Smirnov(K-S) test, p-value<0.001; B. distribution of transformed salivary IL-8 (= $\log_{10}$[1+salivary (IL-8)]), K-S test, p-value=0.358

Appendix

Table 1 Output

One-Sample Kolmogorov-Smirnov Test

		Age
N		623
Normal Parameters[as]	Mean	62.07
	Std. Deviation	9.209
Most Extreme Differences	Absolute	.071
	Positive	.069
	Negative	-.071
Kolmogorov-Smirnov Z		1.780
Asymp. Sig. (2-tailed)		.004

a. Test distribution is Normal.

Ranks

	Perio_CDC_3G	N	Mean Rank
Age	normal	239	292.29
	moderate	267	317.35
	severe	117	340.05
	Total	623	

Test Statistics[a,b]

	Age
Chi-Square	5.949
df	2
Asymp. Sig.	.051

a. Kruskal Wallis Test
b. Grouping Variable: Perio_CDC_3G

성별 * Perio_CDC_3G Crosstabulation

			Perio_CDC_3G			
			normal	moderate	severe	Total
성별	남자	Count	63	117	59	239
		% within 성별	26.4%	49.0%	24.7%	100.0%
		% within Perio_CDC_3G	26.4%	43.8%	50.4%	38.4%
		% of Total	10.1%	18.8%	9.5%	38.4%
	여자	Count	176	150	58	384
		% within 성별	45.8%	39.1%	15.1%	100.0%
		% within Perio_CDC_3G	73.6%	56.2%	49.6%	61.6%
		% of Total	28.3%	24.1%	9.3%	61.6%
Total		Count	239	267	117	623
		% within 성별	38.4%	42.9%	18.8%	100.0%
		% within Perio_CDC_3G	100.0%	100.0%	100.0%	100.0%
		% of Total	38.4%	42.9%	18.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	25.127[a]	2	.000
Likelihood Ratio	25.658	2	.000
Linear-by-Linear Association	23.292	1	.000
N of Valid Cases	623		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 44.88.

One-Sample Kolmogorov-Smirnov Test

		자연치(28)
N		623
Normal Parameters[a]	Mean	21.9952
	Std. Deviation	6.17314
Most Extreme Differences	Absolute	.175
	Positive	.165
	Negative	-.175
Kolmogorov-Smirnov Z		4.360
Asymp. Sig. (2-tailed)		.000

a. Test distribution is Normal.

Ranks

	Perio_CDC_3G	N	Mean Rank
자연치(28)	normal	239	347.22
	moderate	267	307.81
	severe	117	249.63
	Total	623	

Test Statistics[a,b]

	자연치(28)
Chi-Square	23.573
df	2
Asymp. Sig.	.000

a. Kruskal Wallis Test
b. Grouping Variable: Perio_CDC_3G

One-Sample Kolmogorov-Smirnov Test

		비자극성 타액 분비율 (분당 타액 분비율)
N		623
Normal Parameters[a]	Mean	.15087
	Std. Deviation	.13455
Most Extreme Differences	Absolute	.153
	Positive	.153
	Negative	-.148
Kolmogorov-Smirnov Z		3.831
Asymp. Sig. (2-tailed)		.000

a. Test distribution is Normal.

Ranks

	Perio_CDC_3G	N	Mean Rank
비자극성 타액 분비율 (분당 타액 분비율)	normal	239	294.82
	moderate	267	326.26
	severe	117	314.56
	Total	623	

Test Statistics[a,b]

	비자극성 타액 분비율 (분당 타액 분비율)
Chi-Square	3.878
df	2
Asymp. Sig.	.144

a. Kruskal Wallis Test
b. Grouping Variable: Perio_CDC_3G

SMOKING_2G * Perio_CDC_3G Crosstabulation

Count

		Perio_CDC_3G			
		normal	moderate	severe	Total
SMOKING_2G	no	189	183	75	447
	yes	10	34	19	63
Total		199	217	94	510

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.436[a]	2	.000
Likelihood Ratio	19.035	2	.000
Linear-by-Linear Association	16.396	1	.000
N of Valid Cases	510		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 11.61.

DRINK_2G * Perio_CDC_3G Crosstabulation

Count

		Perio_CDC_3G			
		normal	moderate	severe	Total
DRINK_2G	no	115	118	48	281
	yes	124	149	69	342
Total		239	267	117	623

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.751[a]	2	.417
Likelihood Ratio	1.754	2	.416
Linear-by-Linear Association	1.740	1	.187
N of Valid Cases	623		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 52.77.

대사증후군 여부 (IDF_2005) * Perio_CDC_3G Crosstabulation

Count

		Perio_CDC_3G			Total
		normal	moderate	severe	
대사증후군 여부 (IDF_2005)	no	144	182	79	405
	yes	95	85	38	218
Total		239	267	117	623

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.872[a]	2	.144
Likelihood Ratio	3.849	2	.146
Linear-by-Linear Association	2.706	1	.100
N of Valid Cases	623		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 40.94.

edu_2G * Perio_CDC_3G Crosstabulation

Count

		Perio_CDC_3G			Total
		normal	moderate	severe	
edu_2G	1.00	210	235	111	556
	2.00	29	32	6	67
Total		239	267	117	623

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.754[a]	2	.093
Likelihood Ratio	5.550	2	.062
Linear-by-Linear Association	3.057	1	.080
N of Valid Cases	623		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 12.58.

Table 2 Output

Descriptives

LogIG10IL8

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
normal	239	2.6048	.70193	.04540	2.5153	2.6942	.06	4.22
moderate	267	2.5392	.63609	.03893	2.4626	2.6159	.00	3.72
severe	117	2.4838	.60655	.05608	2.3727	2.5948	.25	4.22
Total	623	2.5539	.65741	.02634	2.5022	2.6057	.00	4.22

ANOVA

LogIG10IL8

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.251	2	.626	1.449	.235
Within Groups	267.567	620	.432		
Total	268.818	622			

Estimates

Dependent Variable: LogIG10IL8

Perio_CDC_3G	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
normal	2.594[a]	.042	2.512	2.677
moderate	2.545[a]	.039	2.468	2.622
severe	2.492[a]	.060	2.375	2.610

a. Covariates appearing in the model are evaluated at the following values: Age = 62.07, 성별 = 1.62, 자연치(28) = 21.9952, 비자극성 타액 분비율 (분당 타액 분비율) = .15087, SMEAN(SMOKING_2G) = 1.1235, DRINK_2G = 1.5490, 대사증후군 여부 (IDF_2005) = .3499, edu_2G = 1.1075.

Univariate Tests

Dependent Variable: LogIG10IL8

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Contrast	.796	2	.398	.982	.375	.003	1.964	.221
Error	248.121	612	.405					

The F tests the effect of Perio_CDC_3G. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Table 3 Output

Estimates

Dependent Variable: LogIG10IL8

Perio_CDC_3G	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
normal	2.450[a]	.077	2.297	2.602
moderate	2.512[a]	.056	2.402	2.623
severe	2.461[a]	.080	2.303	2.619

a. Covariates appearing in the model are evaluated at the following values: Age = 64.08, 자연치(28) = 21.7322, 비자극성 타액 분비율 (분당 타액 분비율) = .18385, SMEAN(SMOKING_2G) = 1.2764, DRINK_2G = 1.7531, 대사증후군 여부 (IDF_2005) = .2510, edu_2G = 1.1423.

Univariate Tests

Dependent Variable: LogIG10IL8

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Contrast	.196	2	.098	.268	.765	.002	.536	.092
Error	83.964	229	.367					

The F tests the effect of Perio_CDC_3G. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Estimates

Dependent Variable: LogIG10IL8

Perio_CDC_3G	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
normal	2.659[a]	.050	2.561	2.758
moderate	2.561[a]	.054	2.455	2.668
severe	2.507[a]	.087	2.335	2.679

a. Covariates appearing in the model are evaluated at the following values: Age = 60.82, 자연치(28) = 22.1589, 비자극성 타액 분비율 (분당 타액 분비율) = .13034, SMEAN(SMOKING_2G) = 1.0284, DRINK_2G = 1.4219, 대사증후군 여부 (IDF_2005) = .4115, edu_2G = 1.0859.

Univariate Tests

Dependent Variable: LogIG10IL8

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Contrast	1.306	2	.653	1.502	.224	.008	3.004	.320
Error	162.611	374	.435					

The F tests the effect of Perio_CDC_3G. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Estimates

Dependent Variable: LogIG10IL8

Perio_CDC_3G	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
normal	2.536[a]	.061	2.415	2.656
moderate	2.494[a]	.060	2.376	2.612
severe	2.490[a]	.102	2.290	2.690

a. Covariates appearing in the model are evaluated at the following values: 성별 = 1.69, 자연치(28) = 24.7336, 비자극성 타액 분비율 (분당 타액 분비율) = .16131, SMEAN(SMOKING_2G) = 1.1126, DRINK_2G = 1.5985, 대사증후군 여부 (IDF_2005) = .3282, edu_2G = 1.1429.

Univariate Tests

Dependent Variable: LogIG10IL8

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Contrast	.104	2	.052	.136	.873	.001	.271	.071
Error	95.760	249	.385					

The F tests the effect of Perio_CDC_3G. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Estimates

Dependent Variable: LogIG10IL8

Perio_CDC_3G	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
normal	2.644[a]	.058	2.530	2.759
moderate	2.581[a]	.053	2.478	2.685
severe	2.492[a]	.076	2.343	2.640

a. Covariates appearing in the model are evaluated at the following values: 성별 = 1.56, 자연치(28) = 20.0467, 비자극성 타액 분비율 (분당 타액 분비율) = .14344, SMEAN(SMOKING_2G) = 1.1313, DRINK_2G = 1.5137, 대사증후군 여부 (IDF_2005) = .3654, edu_2G = 1.0824.

Univariate Tests

Dependent Variable: LogIG10IL8

	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Contrast	1.076	2	.538	1.252	.287	.007	2.503	.272
Error	152.115	354	.430					

The F tests the effect of Perio_CDC_3G. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Figure 1 Output

One-Sample Kolmogorov-Smirnov Test

		Sal_IL-8_pg/mL
N		623
Normal Parameters[a]	Mean	967.28154
	Std. Deviation	1978.52416
Most Extreme Differences	Absolute	.312
	Positive	.248
	Negative	-.312
Kolmogorov-Smirnov Z		7.799
Asymp. Sig. (2-tailed)		.000

a. Test distribution is Normal.

One-Sample Kolmogorov-Smirnov Test 2

		LogIG10IL8
N		623
Normal Parameters[a]	Mean	2.5539
	Std. Deviation	.65741
Most Extreme Differences	Absolute	.037
	Positive	.023
	Negative	-.037
Kolmogorov-Smirnov Z		.926
Asymp. Sig. (2-tailed)		.358

a. Test distribution is Normal.

국문초록

1. 연구목적

치주병은 만성 염증성 질환으로 구강내 병원균과 숙주의 방어기전의 균형파괴로 인해 발생되는 질병이다. 몇몇 연구들에 따르면 치은열구액이나 혈청의 interleukin-8 (IL-8)은 치주병과 연관관계가 있다. 하지만 타액의 IL-8과 치주병의 연관성에 대한 연구는 부족하다. 이 연구의 목적은 치주병과 타액내 IL-8과의 연관성을 알아보는 것이다.

2. 연구방법

양평 건강 코호트 조사대상자 중에서 623명이 선정되었다. 치주병의 진단은 치과의사가 Centers for Disease and Control/American Academy of Periodontology (CDC/AAP) 기준을 이용하여 치조골 소실을 파노라마 방사선 사진상에서 평가하여 수행되었다. IL-8은 multiplexed bead immunoassay (Luminex)를 이용하여 측정하였다. 나이, 성별, 흡연여부, 음주여부, 교육수준에 대한 정보는 인터뷰를 통해 얻었다. 분산분석(ANOVA)을 이용하여 치주병 집단간 타액 내 로그변형된 IL-8의 차이를 비교하였고, 혼동변수들을 보정하여 공분산분석(ANCOVA)을 시행하여 그룹간 로그변형된 IL-8을 비교하였다.

3. 결과

치주병의 심도와 타액내 IL-8 사이에서 통계적인 유의성이 나타나지 않았다 (ANOVA, p-value=0.235). 혼동변수들을 보정한 다음에도, 통계적인 유의성은 관찰되지 않았다. (ANCOVA, p-value=0.375). 성별과 나이에 따른 그룹간 계층분석 결과에서도 유의성은 나타나지 않았다.

4. 결론

치주병과 타액내 IL-8은 유의한 연관성이 없었다. 이 결과를 확증하기 위한 후속 연구가 필요할 것으로 사료되었다.

주요어: 치주병, 인터루킨-8, 타액, 분자역학

학번: 2012-22157