Atherogenic Biomarkers and Gingival Bleeding Among Smokers

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Abstract

Objectives: Smoking is a significant risk factor for gingivitis and has detrimental effects on both oral health and the cardiovascular system. This study aimed to evaluate the association between cardiovascular biomarkers and gingival bleeding among smokers.

Materials and Methods: This cross-sectional study comprising 60 smokers (mean age, 59.9±13.7 years) was conducted at an outpatient smoking cessation clinic. The smokers were divided into two groups based on the presence or absence of gingival bleeding, which was assessed by probing. α1-antitrypsin low-density lipoprotein complex (AT-LDL), an oxidatively modified LDL complex, causes progressive atherosclerosis. The clinical characteristics and blood markers including AT-LDL levels were measured in these patients.

Results: Significantly higher (p=0.03) levels of AT-LDL, an oxidized LDL complex that promotes atherosclerosis, were observed among smokers with no gingival bleeding on probing when compared to that among smokers with gingival bleeding. The pocket depths in smokers without gingival bleeding were significantly (p=0.04) lower than those among smokers with gingival bleeding.

Conclusion: The absence of gingival bleeding among smokers was associated with higher levels of AT-LDL. These findings could indicate reduced blood flow due to atherosclerosis among smokers with no gingival bleeding.

Keywords: Smokers, gingival bleeding, atherosclerosis, LDL, AT-LDL
Introduction

Smoking is associated with various major cardiovascular risk factors that can exhibit detrimental effects on the heart and blood vessels. It is known to cause chronic and irreversible changes related to coronary atherosclerosis\(^1\). Generally, a single puff of a cigarette consists of \(1 \times 10^{17}\) oxidant molecules that can cause oxidative stress\(^2\). The oxidative stress can be assessed by directly measuring the production of reactive oxygen species in the peripheral blood cells or by measuring the amounts of lipid peroxidation products and oxidized proteins on the target molecules. Low-density lipoproteins (LDL) are more vulnerable to oxidization among smokers compared to non-smokers due to the higher levels of reactive oxygen species in the former\(^2\). Oxidized LDL stimulates endothelial cell toxicity and vasoconstriction\(^3\), and \(\alpha_1\)-antitrypsin low-density lipoprotein complex (AT-LDL) is an oxidatively modified LDL complex that contributes to advanced atherosclerosis. AT-LDL is found in human serum and atheromatous plaque, and the circulatory levels of AT-LDL appear to mirror the activity of foam cells in atherosclerotic lesions. Hence, AT-LDL levels are thought to be linked to oxidative stress and atherosclerosis caused by smoking\(^4,5\). Our previous study demonstrated a decrease in AT-LDL levels after 3 months of smoking cessation and a further decrease after one year of smoking cessation; therefore, it could be considered as a valuable marker for oxidative stress among smokers\(^4\).

Serum amyloid A-LDL complex (SAA-LDL) is another inflammatory marker viewed as an oxidatively denatured form of LDL. Higher levels of circulating SAA-LDL were found to be associated with a higher susceptibility to atherosclerotic events\(^6\). In addition, a study by Wada et al.\(^5\) reported that higher SAA-LDL levels were associated with a longer duration of smoking.

Gingival health can be assessed by examining the gingival blood flow and gingival crevicular fluid. A study demonstrated an association between smoking and gingival health. An increase in gingival blood flow and gingival crevicular fluid was reported within a week after smoking cessation indicating recovery of the gingival microcirculation\(^7\). One study suggested that smoking may lead to reduced gingival bleeding due to alterations in the blood vessels of the periodontium\(^8\).

Smokers exhibit an 80% higher risk of presenting with periodontitis than non-smokers and quitters\(^9\). Periodontitis is an inflammatory disease of the supporting tissues of the teeth; it induces progressive destruction of the periodontal ligament, resulting in periodontal pocket formation, gingival bleeding, and alveolar bone loss\(^10\). A recent questionnaire-based study found a significant association between periodontal disease and a known family history of periodontal disorders as well as the smoking duration\(^11\). Several previous studies support the association between periodontal disease and cardiovascular disorders\(^12,13\). A global clinical trial found positive associations between the risk factors and biomarkers of cardiovascular diseases, tooth loss, and self-reported gum bleeding\(^13\). Smokers demonstrate a unique characteristic of presenting with gingivitis in association with a reduced blood flow\(^7,8\).

However, to the best of our knowledge, the association between cardiovascular risk factors and gingivitis among smokers has not been reported so far.

The current study aimed to investigate the association between cardiovascular biomarkers and gingival bleeding among smokers.

Materials and Methods

Study Population

This cross-sectional study was conducted among 83 dentate smokers who visited the National Hospital Organization Kyoto Medical Centre outpatient clinic between January 18, 2017, and October 3, 2018, and reported a desire for smoking cessation. Those who provided written informed consent were included in the study, whereas those with advanced cancer (requiring palliative care), patients undergoing pharmacotherapy or any anticoagulation therapy for cardiovascular conditions and those in whom the gingival bleeding could not be assessed due to missing teeth were excluded.
The age, number of cigarettes per day, smoking years, Fagerström test of nicotine dependence (FTND) score, body mass index, waist circumference, blood pressure, and respiratory carbon monoxide (CO) levels were recorded. The FTND is a standard instrument consisting of items that are used to assess the intensity of the physical addiction to nicotine\(^{(14)}\). The items are summed to yield a total score of 0-10. The higher the FTND score, the more intense the patient’s physical dependence on nicotine\(^{(14)}\).

Blood was collected from the antecubital vein 2-3 h after a meal to determine the high-density lipoprotein (HDL-C), LDL-C, hemoglobin A1c (HbA1c), C-reactive protein (CRP), triglycerides (TG), SAA-LDL, and AT-LDL levels.

**Periodontal Examinations**

Clinical examination of the oral cavity was conducted using a mouth mirror and a calibrated periodontal probe. A single experienced dentist recorded the clinical parameters throughout the study using the same instruments. A calibrated periodontal probe with controlled force was used to assess the gingival bleeding to avoid trauma and false-positive bleeding from healthy tissues. The presence or absence of gingival bleeding was assessed. Gentle probing was performed by running a probe around the teeth up to a depth of 2 mm in the sulcus without applying any force apically. The WHO periodontal probe developed by the Japanese company YDM Corporation was used to assess the periodontal status of the patient; a recommended probing force of 20-25 g was used\(^{(15)}\). The periodontal pocket depth was classified into 3 groups: grade 0 [periodontal depth (PD)-0-3 mm], grade 1 (PD-4 to 5 mm) and grade 2 (PD>6 mm).

**Statistical Analysis**

The Statistical Package for Social Sciences (SPSS) Statistics, version 17.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The prevalence and association between the various parameters were evaluated. Comparisons between smokers with and without bleeding on probing were made using the Fisher’s exact test, unpaired t-test, and Mann-Whitney U test. Data are presented as mean ± standard deviation for normally distributed data and as median (interquartile range) for data that were not normally distributed.

**Results**

The cross-sectional data of 83 smokers collected during the first visit to the smoking cessation clinic were analyzed. However, only 60 patients were included in this study due to the non-availability of data from the remaining patients (Figure 1).

For comparison purposes, the smokers were divided into two groups based on the presence or absence of gingival bleeding (Table 1). Table 1 shows the characteristics of the patients in the two groups. No significant differences in age, amount of smoking, years of smoking, FTND score, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, and respiratory CO levels were observed between the two groups. Furthermore, no significant differences were observed between a group with gingival bleeding and the group without gingival bleeding in the blood markers such as HbA1c, TG, creatinine, CRP, and SAA-LDL. The LDL-C level tended to be higher, and the HDL-C level tended to be lower among smokers with gingival bleeding, statistical significance notwithstanding (Table 1). The neutrophil-lymphocyte ratio, a marker of inflammation, tended to be higher among smokers without gingival bleeding when compared to that in smokers with gingival bleeding (p=0.12). AT-LDL showed a significant positive association (p=0.03), indicating that smokers with no

![Figure 1. Flow chart of the study participants](image-url)
gingival bleeding exhibited higher levels of AT-LDL than those with gingival bleeding.

As shown in Table 1, a significantly fewer number of smokers without gingival bleeding presented with grade 1 and grade 2 pocket depths when compared to those with gingival bleeding (p=0.04); alternatively, the number of smokers with grade 0 pockets was higher among those without gingival bleeding. However, no association between the amount of smoking and the number of smoking years was observed.

**Discussion**

The purpose of the present study was to investigate the association between atherosclerotic biomarkers and gingival bleeding in smokers. Both SAA-LDL and AT-LDL are atherosclerotic factors; however, AT-LDL has been strongly associated with smoking. AT-
LDL levels have been linked to smoking habits, and a rapid decrease in these levels has been observed after smoking cessation, suggesting its role as a valuable biomarker of oxidative stress in smokers\(^5\). In the present study, the absence of gingival bleeding among smokers was associated with higher levels of AT-LDL. Additionally, tendencies toward an increase in LDL-C levels, decrease in HDL-C levels, and increase in the neutrophil-lymphocyte ratio were observed among smokers without gingival bleeding, thereby indicating the profile of the atherogenic lipoproteins in these patients. Smoking might reduce gingival bleeding owing to changes in the proportion of the blood vessels in periodontal tissues\(^8\).

A global study by Vedin et al.\(^{13}\) among never (31%), current (18%), and former smokers (51%) concluded that gingival bleeding was associated with a higher risk of cardiovascular disease. However, the majority of the subjects in that study were non-smokers, and the gingival bleeding was self-reported. Another study by Tamaki et al.\(^{12}\) comprising 22 never-smokers with chronic periodontitis (30% of the sites in the oral cavity presented with gingival bleeding) reported higher levels of circulating oxidized LDL and oxidative stress among the subjects with chronic periodontitis compared to healthy patients. The discrepancies in these findings between the aforementioned studies and the current study may be due to differences in smoking status.

Gingival blood flow and crevicular fluid are well-known indicators of gingival health. Nicotine induces vasoconstriction and slows healing following periodontal therapy. Morozumi et al.\(^7\) reported a considerable increase in gingival blood flow 3 days after smoking cessation. Gingival blood flow and gingival crevicular fluid act to improve the gingival microcirculation and periodontal health. Reduced inflammatory characteristics in the gingiva of smokers are related to a reduction in the number of inflammatory cells\(^{16}\). Smoking can drastically change the typical appearance of gingivitis and periodontitis by suppressing the signs of inflammation.

As a result, less gingival bleeding among smokers does not always indicate a healthy gingiva. No gingival bleeding among smokers might reflect atherosclerosis\(^{16}\). Therefore, distinguishing between healthy gingiva and the absence of gingival bleeding in smokers is difficult, which could lead to difficulties in diagnosing gingivitis despite deterioration in the condition.

Healthcare providers, such as dentists, are the first points of contact for the oral health check-up of patients and are ideally supposed to take the smoking history of the patient. Patients with no gingival bleeding can be prone to atherosclerotic changes and must be treated accordingly and with more vigilance. The patients can be referred to a physician for further check-ups to evaluate the presence of other health conditions, such as cardiovascular diseases. However, the results of the present study do not indicate that smokers with presence of gingival bleeding are not at risk of cardiovascular events.

Our previous study\(^{17}\) demonstrated an increase in periodontal pocket depth and gingival bleeding after 3 months of smoking cessation, thus indicating that the depth of the periodontal pocket increases with the increase in gingival flow, and these could be a part of the healing process. Therefore, reduced or no gingival bleeding with shallow pockets among smokers does not indicate healthy gingiva. The outcomes of the current and previous studies will be valuable in providing advice to patients who are undergoing smoking cessation programs. However, the dentists must advise their patients that quitting the smoking habit could cause an increase in gingival bleeding and pocket depth, initially.

This study has some limitations. The number of patients included in the study was limited. Moreover, causal interpretations were not possible, owing to the cross-sectional nature of the study. Additional studies using a significantly higher number of patients are required to observe the association between smokers and the AT-LDL levels.
Conclusion

The absence of gingival bleeding among smokers was associated with higher levels of AT-LDL, which could be connected with atherogenic events. The findings of this study indicated a reduction in blood flow due to atherosclerosis among smokers without gingival bleeding. These findings can be used by healthcare providers, particularly dentists, to assess and interpret correctly the dental health of smokers and, if necessary, recommend them to physicians to prevent the development of cardiovascular issues in the future.

Ethics

Ethics Committee Approval: Ethics approval for the study was obtained from the Ethical Review Committee at the National Hospital Organization Kyoto Medical Centre (Fushimi-Ku, Kyoto, Japan) (approval no.: - date: 14-042).

Informed Consent: A written informed consent was provided by all the patients who participated in the study.

Authorship Contributions


Conflict of Interest: The authors declare no conflicts of interest concerning the authorship or publication of this article.

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