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Association between passive and active smoking evaluated by salivary cotinine and periodontitis

Yumiko Yamamoto[1], Nobuko Nishida[1,2], Muneo Tanaka[1], Naoji Hayashi[1], Ryoichi Matsuse[3], Kunio Nakayama[4], Kanehisa Morimoto[4] and Satoshi Shizukuishi[1]

[1]Department of Preventive Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan; [2]Japan Foundation for Aging and Health; [3]Kyoto Medical Science Laboratory, Inc., Kyoto, Japan; [4]Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

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Abstract

Aim: This study attempted to determine the relationship between passive and active smoking on the basis of salivary cotinine levels and periodontitis severity.

Methods: Japanese workers ($n = 273$) were surveyed via an oral examination, a self-administered questionnaire and collection of whole saliva. Probing pocket depth (PPD) and clinical attachment level (CAL) served as periodontal parameters. Periodontitis was defined as the presence of two or more teeth with PPD $\geqslant 3.5$ mm and CAL $\geqslant 3.5$ mm. Salivary cotinine was determined using ELISA. Statistical methods included Wilcoxon's rank-sum test and multiple logistic regression analysis.

Results: Based on the results of receiver-operating characteristic plots for cotinine-level classification derived from self-reported smoking status, non-, passive and active smokers were defined as those subjects exhibiting cotinine levels of 0, 1–7 and $\geqslant 8$ ng/ml, respectively. Numbers of teeth displaying CAL $\geqslant 3.5$ mm in passive and active smokers were significantly higher than those in non-smokers. Multiple logistic regression analysis revealed significantly higher periodontitis odds ratios in passive and active smokers relative to non-smokers following adjustment for other lifestyle factors; odds ratios were 2.87 [95% confidence interval (CI); 1.05–7.82] and 4.91 (95% CI; 1.80–13.35), respectively.

Conclusion: These findings suggest that passive smoking classified in terms of salivary cotinine level may be an independent periodontitis risk indicator.

Key words: active smoking; cotinine; passive smoking; periodontitis; saliva

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Smoking is generally accepted as a major environmental risk factor of periodontal diseases. The majority of previous investigations examined the association between periodontitis and active smoking (Johnson & Hill 2004). A meta-analysis of six studies concluded that active smokers were nearly three times more likely to exhibit severe periodontitis in comparison with non-smokers (Papapanou 1996). Approximately 40% of periodontitis cases are thought to be attributable to active smoking based on data from the National Health and Nutrition Examination Survey (NHANES) III (Tomar & Asma 2000). We previously demonstrated that active smoking displays the greatest impact on periodontitis among lifestyle-related factors (Nishida et al. 2004, 2005). Recently, Arbes et al. (2001) reported that adjusted odds of periodontal disease were 1.6 times greater for persons exposed to passive smoke than for persons not exposed via evaluation of self-reported environmental tobacco smoke (ETS) exposure. This result suggested the presence of a harmful effect in connection with passive smoking with respect to periodontal disease.

In most investigations, smoking status was evaluated exclusively via a self-administered questionnaire. The validity of self-reported smoking is often questioned because of the widespread belief that smokers are inclined to underestimate the amount smoked or to deny smoking altogether (Patrick et al. 1994). In addition, self-reported exposure to ETS may require detailed questionnaire items (Jarvis et al. 1984). Cotinine, a major metabolite of nicotine in body fluids, is considered an accurate indicator of current smoking or of exposure to smoke. Nicotine possesses a very short half-life in the blood, approximately 2 h; in contrast, cotinine exhibits a longer serum half-life, approximately 19 h (Haley et al. 1983). Thus, cotinine has been employed as a chemical

marker of nicotine exposure in numerous studies relating smoking to disease (Istavan et al. 1994). However, few reports have documented an association between cotinine level in body fluids and periodontitis (McGuire et al. 1989, Gonzalez et al. 1996, Chen et al. 2001). Furthermore, a correlation between passive smoke exposure determined with respect to cotinine level and periodontitis has not been used. The objective of the present study was to characterize the relationship between passive and active smoking on the basis of salivary cotinine levels and severity of periodontitis.

Subjects and Methods

Study population

Three hundred and sixty Japanese factory workers employed at a manufacturing company in Osaka were available for evaluation. In 2003, 273 (75.8%) of these individuals (236 males and 37 females, aged 18–62 years) were surveyed via an oral examination, a self-administered questionnaire and collection of whole saliva. Oral status was not examined in 61 subjects because of reasons corresponding to their work; additionally, 26 participants refused to provide saliva. Two hundred and fifty-six workers (221 males and 35 females, aged 18–62 years) completed all items of a self-administered questionnaire. Informed consent was obtained from all subjects. Permission for this study was obtained from the Ethical Committee for Clinical Research of Osaka University Graduate School of Dentistry.

Assessment of lifestyle-related factors

Lifestyle behaviour was evaluated in terms of eight categories (cigarette smoking, alcohol consumption, sleeping hours, breakfast, nutritional balance, working hours, physical exercise and mental health) utilizing a protocol developed by Morimoto (Kusaka et al. 1992, Shizukuishi et al. 1998). Questions were presented in multiple-choice format (from two to six possible answers). Each answer was dichotomized as a ''good'' or ''not good'' health practice. Body mass index (BMI) was calculated as an indicator of overall adiposity with regard to obesity. BMI was computed from weight in kilograms divided by square height in metres.

Assessment of smoking behaviour

Data corresponding to smoking behaviour (never, past or current smoker) were derived from a self-administered questionnaire. Moreover, individual passive smoking situation was probed in the self-administered questionnaire: ''Are you currently exposed to tobacco smoke from other people within a week?'' Five independent locations were examined: home, workplace, restaurants, recreation halls and traffic stations. Additionally, the frequency of tobacco exposure at four levels with respect to each of the aforementioned locations was surveyed: almost every day, sometimes, not at all and uncertain. The questionnaire was based on the guidelines of the Survey of Smoking and its Effect on Health in Japan (Ministry of Health, Labour and Welfare, Japan, 1999). ETS score was calculated on the basis of this self-reported questionnaire to evaluate passive smoking status as follows: the score for ''almost every day'' was 2, the score for ''sometimes'' was 1, the score for ''not at all'' was 0 and the score for ''uncertain'' was 0.5. Scores for the five locations were totalled and the individual ETS score was obtained. Subjects with or without ETS exposure were defined as those participants displaying ETS scores >2 or $\leqslant 2$, respectively.

Assessment of salivary cotinine level

Subjects received a piece of paraffin gum at the annual health check-up; subsequently, following chewing, saliva samples were obtained by expectoration. First, participants were asked to chew a piece of paraffin gum for 30 s. Then, they were asked to spit approximately 2.0 ml of saliva into a test tube. Saliva samples were collected between 9 and 12 am. Samples, which were stored at -80°C until use, were delivered to the laboratory for cotinine analysis. Cotinine levels were measured via a competitive enzyme-linked immunosorbent assay (ELISA). ELISA plates (Nunc A/S, Roskilde, Denmark) were coated (0.1 ml/well) with a solution of rabbit polyclonal anti-goat IgG (10 μg/ml) (Dako Cytomation A/S, Glostrup, Denmark) in tris-buffer, pH 8.4, and incubated overnight at 4°C. The plates were blocked with 0.2 ml of 10 mM phosphate buffer, pH 7.5, containing 0.1% BSA (phosphate-BSA buffer); subsequently, plates were incubated for 1 h at room temperature and stored at 4°C. A standard inhibition curve was generated by serial dilution (1:2) of a solution consisting of cotinine (160 ng/ml) in phosphate-BSA buffer to obtain seven dilutions of known concentration. Each dilution was tested in duplicate via addition of 50 μl of cotinine solution, 50 μl of (1/10,000) goat polyclonal anti-cotinine reagent (Affiniti Research Product Ltd, Exeter, UK) and 50 μl of cotinine conjugated with horseradish peroxidase, which was derived from carboxyl-cotinine (Aldrich Chem Co., Milw., WI, USA) and horseradish peroxidase (Sigma Co., St Louis, MO, USA), as described previously by Grabarek and Gergely (1990). Each unknown sample was also tested in duplicate with 50 μl of saliva at 1:2 dilution and 50 μl of anti-cotinine reagent and horseradish-conjugated cotinine reagent. Following a 1-h incubation at 25°C, plates were washed three times with 0.3 ml of distilled water. A substrate solution (100 μl) containing tetramethylbenzidine (TMB) was then added, and plates were incubated for 30 min at 25°C in the dark. Colour development was terminated upon the introduction of 100 μl (1 M) of phosphoric acid. The optical density of each well was determined with a microplate reader at 450 nm. The minimum limitation of the measurement for salivary cotinine was 1 ng/ml in this study. The coefficients of variation of the assay were 5.8% within batch and 9.6% between batches.

Assessment of periodontitis

The periodontal condition, measured as probing pocket depth (PPD) and clinical attachment level (CAL) in millimetres, was recorded using an automated probe (Vivacare TPS Probe™, Schaan, Liechtenstein) involving a constant force (20 g) by three examiners. Probing was performed at six sites per tooth for all teeth (excluding the third molar); moreover, the deepest reading was recorded for each. In two selected quadrants – one maxillary and one mandibular – CAL was calculated based on the probed distances (in millimetres) from the free gingival margin to the cemento-enamel junctions and the base of the sulcus; the greatest CAL was recorded for each tooth. Subsequently, subjects were classified into two groups, periodontitis or non-periodontitis, based on placement above or below each two teeth characterized by PPD $\geqslant 3.5$ mm and CAL

$\geqslant$3.5 mm, respectively. The disease group may demonstrate moderate/severe periodontitis (Armitage 1999). Calibrated examiners conducted the periodontal examinations. The mean κ values among the examiners were 0.71 and 0.77 for assessment of PPD and CAL, respectively, when PPD or CAL of 3.5 mm served as the cut-off point.

Statistical analysis

Data were analysed with a statistical package (Stat View, SAS Institute, Cary, NC; SPSS 10.0J, SPSS Inc., Chicago). To examine the effectiveness of salivary cotinine level as an indicator of tobacco smoke exposure, receiver-operating characteristic (ROC) plots were generated and analysed (Zar 1996). The Kruskal–Wallis and Wilcoxon rank-sum tests were utilized to evaluate differences in periodontal status among the three smoking types, which were determined by a self-administered questionnaire or salivary cotinine levels. Multiple logistic regression analysis was used with respect to consideration of other confounding factors such as age and to determine which lifestyle variables demonstrated a significant effect on periodontitis. Odds ratios and their 95% confidence intervals (CI) were also calculated. Data, which were adjusted initially for age alone, were subsequently adjusted for the following multiple covariates: age, sex, alcohol consumption and BMI. All reported *p*-values are two-tailed; moreover, those *p*-values less than 0.05 were considered statistically significant.

Results

Periodontal status of subjects was characterized based on the number of teeth exhibiting PPD$\geqslant$3.5 mm and CAL$\geqslant$3.5 mm. The mean ($\pm$ SE) numbers of teeth with PPD$\geqslant$3.5 mm and CAL$\geqslant$3.5 mm were 4.7 ($\pm$ 0.3) and 1.6 ($\pm$ 0.1), respectively. The number of teeth displaying PPD $\geqslant$3.5 mm varied from a low of 0 to a high of 25, whereas the number of teeth with CAL $\geqslant$3.5 mm varied from a low of 0 to a high of 11.

In order to assess exposure to cigarette smoke, self-reported questionnaires and salivary cotinine levels were examined. Subjects were categorized into three groups via a self-reported questionnaire related to smoking behaviour: current smokers and non-current smokers with and without ETS exposure (Table 1). The mean cotinine level of current smokers was 145 ng/ml; moreover, current smokers displayed significantly higher cotinine levels in comparison with non-current smokers. Furthermore, current smokers exhibited significantly greater numbers of teeth with PPD$\geqslant$3.5 mm relative to non-current smokers. However, no meaningful difference in the number of teeth characterized by PPD$\geqslant$3.5 mm or CAL $\geqslant$3.5 mm was observed between non-current smokers with and without ETS exposure.

ROC curves were analysed to determine whether self-reported smoking status could be assessed via the salivary cotinine test (Fig. 1). The area under the ROC plots was 0.983 upon application of the cotinine test for identification of current and non-current smokers (Fig. 1a); moreover, sensitivity and specificity displayed maximum readings of 0.968 and 0.975, respectively, for the cut-off value of 8 ng/ml. On the other hand, when the cotinine test was utilized to identify non-current smokers with and without ETS exposure, the area under the ROC plots was 0.528 (Fig. 1b). This result indicated that evaluation of self-reported EST exposure may not be possible with the salivary cotinine test. Consequently, non-smokers, passive smokers and active smokers were defined as those subjects characterized by cotinine levels of 0, 1–7 and $\geqslant$8 ng/ml, respectively.

The mean cotinine levels of active and passive smokers were 143 and 3 ng/ml, respectively (Table 2). In addition, the mean number of teeth exhibiting CAL $\geqslant$3.5 mm in non-smokers was 0.9; in contrast, the mean numbers of teeth characterized by CAL $\geqslant$3.5 mm in active and passive smokers were 1.9 and 1.6, respectively. Each active and passive smoker displayed significantly higher numbers of teeth with CAL $\geqslant$3.5 mm than did non-smokers ($p<0.05$). The mean number of teeth demonstrating PPD$\geqslant$3.5 mm in passive smokers tended to be higher than that in non-smokers; however, no meaningful difference was detected. Subjects were classified into two groups, periodontitis or non-periodontitis, based on placement above or below each two teeth with PPD$\geqslant$3.5 mm and CAL$\geqslant$3.5 mm, respectively. The periodontitis group included 79 individuals (30.9%). Subsequently, multiple logistic analysis of passive and active smokers was conducted in order to evaluate other confounding factors such as age, sex and other lifestyle factors (Table 3). Odds ratios were 2.84 (95% CI: 1.10–7.32) for passive smokers and 5.13 (95% CI: 1.99–13.19) for active smokers in comparison with non-smokers. Additional adjustments for age, sex, alcohol consumption and BMI showed significant correlations; odds ratios were 2.87 (95% CI: 1.05–7.82) for passive smokers and 4.91 (95% CI: 1.80–13.35) for active smokers.

Table 1. Self-reported smoking behaviour, salivary cotinine levels and periodontal status

Classified by self-reported smoking behaviour	*N*	Salivary cotinine levels (ng/ml)			Number of teeth with PPD $\geqslant$3.5 mm			Number of teeth with CAL $\geqslant$3.5 mm		
		mean	SE	mean rank	mean	SE	mean rank	mean	SE	mean rank
Current smokers	95	145	9	206 ***	6.4	0.6	157 **	2.0	0.3	143 *
Non-current smokers				***			***			
With ETS exposure	91	2	0	84	4.0	0.6	115	1.3	0.2	116
Without ETS exposure	70	5	2	81	3.4	0.5	108	1.6	0.3	125
p-Value (Kruskal–Wallis test)				<0.0001			<0.0001			$=0.0274$

*$p<0.01$ (Wilcoxon rank-sum test).
**$p<0.001$ (Wilcoxon rank-sum test).
***$p<0.0001$ (Wilcoxon rank-sum test).
PPD, probing pocket depth; CAL, clinical attachment level; ETS, environmental tobacco smoke.

Discussion

The present investigation assessed the level of smoking exposure based on the concentration of salivary cotinine using a quantitative assay. The saliva flow rate has been shown to affect saliva biomarker concentrations in periodontitis subjects significantly (Brock et al. 2004). In order to neutralize the influence of salivary flow rate to as great an extent as possible, cotinine concentration was adjusted with total protein or inorganic phosphorus; however, these parameters did not provide satisfactory differentiation with respect to smoking status in comparison with cotinine concentration (data not shown). The questionnaire and cotinine data afforded consistent information regarding exposure of active smokers but not exposure of passive smokers. In the ROC analysis, the area under ROC curves displayed very high values; furthermore, when the cut-off point for salivary cotinine (8 ng/ml) was selected, specificity and sensitivity were 0.975 and 0.968, respectively. Additionally, the correlation coefficient between salivary cotinine and cigarette consumption/day was 0.60 ($p < 0.0001$, data not shown). This correlation was similar to those appearing in the literature (Etter et al. 2000). In this study, cotinine concentration that best separated current smokers and non-current smokers (8 ng/ml) was somewhat lower than those levels documented in most previous reports, in which cut-off values ranged mainly between 7 and 20 ng/ml (Patrick et al. 1994, Etter et al. 2000). The average cigarette consumption/day was 19.4 (data not shown), and the mean salivary cotinine level was 145 ng/ml among current smokers in the current investigation. These findings indicated that most participants labelled as smokers were moderate smokers. Thus, this situation would reduce the cut-off value in comparison with other sample populations.

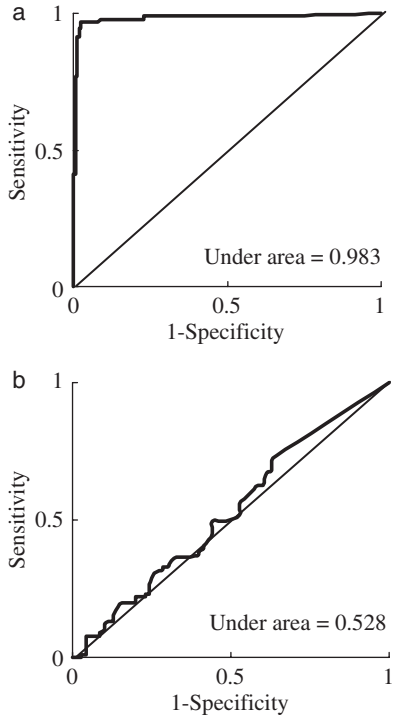

Fig. 1. ROC curves for assessment of salivary cotinine test ability in terms of detection of self-reported smoking status: (A) detection of current smoking status; (B) detection of ETS exposure status.

Most investigators documented increasing cotinine levels with increasing levels of self-reported ETS exposure (Benowitz 1996). However, in the current study, when subjects with or without ETS exposure were defined as those participants displaying ETS scores >2 or $\leqslant 2$, respectively, an association between self-reported exposure to ETS and salivary cotinine concentration was not observed. This association was not apparent despite the fact that the scores for the definition of ETS exposure were changed to $\geqslant 2.0$ or $\geqslant 2.5$ of ETS score (data not shown). Given that smoking may be permitted in the workplace, the majority of passive smokers may be exposed in the workplace but may not recognize ETS exposure. Etzel (1990) noted that passive smokers typically exhibited salivary cotinine concentrations < 10 ng/ml. This observation may support the definition of passive smokers as those subjects characterized by salivary cotinine levels of 1–7 ng/ml. ELISA data revealed a salivary cotinine detection limit of 1 ng/ml in this study; as a result, light passive smokers may be treated as non-smokers in some instances. However, regardless of the assay used, numerous investigations demonstrated meaningful differences in cotinine levels between ETS- and non-exposed populations of non-smokers (Benowitz 1996). The results of ROC analysis do not suggest that salivary cotinine level may be a superior measure of smoking in comparison with self-reporting. In order to justify substitution of biochemical measures of smoking behaviour for self-reported cigarette smoking to quantify risk, correlation with disease outcomes must be demonstrated (Perez-Stable et al. 1995). Periodontal status relative to smoking status classified by both self-reporting and salivary cotinine levels was compared. Although no meaningful difference was observed in periodontal status between non-current smokers with

Table 2. Smoking behaviour classified by salivary cotinine levels and periodontal status

Classified by salivary continine levels	*N*	Salivary cotinine levels (ng/ml)			Number of teeth with PPD $\geqslant$ 3.5 mm			Number of teeth with CAL $\geqslant$ 3.5 mm		
		mean	SE	mean rank	mean	SE	mean rank	mean	SE	mean rank
Active smokers ($\geqslant$8 ng/ml)	102	143	9	223 ***	6.2	0.6	165 **	1.9	0.2	152 **
Passive smokers (1–7 ng/ml)	118	3	0	113 ***	4.5	0.5	127 ***	1.6	0.2	136 *
Non-smokers (0 ng/ml)	53	0	0	27 ***	2.3	0.3	105 ***	0.9	0.3	110 *
p-Value (Kruskal–Wallis test)				< 0.0001			< 0.0001			$= 0.0031$

*$p < 0.05$ (Wilcoxon rank-sum test).
**$p < 0.001$ (Wilcoxon rank-sum test).
***$p < 0.0001$ (Wilcoxon rank-sum test).
PPD, probing pocket depth; CAL, clinical attachment level.

Table 3. Association between periodontitis risk* and smoking status determined by salivary cotinine levels

	Smoking status determined by salivary cotinine levels		
	Non-smokers (0 ng/ml)	Passive smokers (1–7 ng/ml)	Active smokers (⩾8 ng/ml)
Participants (*N*)	48	111	97
Age (mean, years)	38.9	40.6	41.8
Male/female (*N*)	33/15	98/13	92/5
Odds ratio†	1	2.84	5.13
(95% CI)		(1.10–7.32)	(1.99–13.19)
Odds ratio‡	1	2.96	5.18
(95% CI)		(1.11–7.89)	(1.94–13.83)
Odds ratio§	1	2.95	5.16
(95% CI)		(1.10–7.91)	(1.91–13.92)
Odds ratio¶	1	2.87	4.91
(95% CI)		(1.05–7.82)	(1.80–13.35)

*Periodontitis was defined as the presence of two teeth characterized by PPD⩾3.5 mm and CAL⩾3.5 mm.
†Unadjusted.
‡Adjusted for age.
§Adjusted for age and sex.
¶Adjusted for age, sex, alcohol consumption and BMI.
CI, Confidence interval; PPD, probing pocket depth; CAL, clinical attachment level.

and without ETS exposure, passive smokers defined by salivary cotinine displayed significantly more severe periodontal status than non-smokers. Self-reporting measures of ETS exposure are likely imprecise indicators of intake tobacco smoke because of variations in the concentration of tobacco smoke, proximity of non-smokers to smokers, room ventilation and other environmental characteristics. On the other hand, limitations associated with utility of cotinine relate to the lack of a standard measure of long-term ETS exposure; additionally, inter-individual variability exists in cotinine measurements. However, steps are implemented in order to compensate for this variability in studies involving large numbers of subjects, as in epidemiologic studies; furthermore, assumption of a steady state for cotinine levels is reasonable with respect to consideration of daily exposure to ETS in the workplace and/or at home (Benowitz 1996). Therefore, salivary cotinine levels were used to assess smoking status in the present investigation.

Our findings confirmed the relationships between periodontitis and active smoking and passive smoking as determined by salivary cotinine levels. Following adjustment for other lifestyle factors, the odds ratio of active smokers was 4.91 (95% CI: 1.80–13.35). Gonzalez et al. (1996) reported the quantitative association between salivary cotinine levels and clinical parameters including CAL, PPD and bone crest height. Furthermore, serum cotinine level exhibited a direct correlation with outcomes of progressive periodontal breakdown in patients monitored for 1 year (Machtei et al. 1997). In contrast, Chen et al. (2001) noted that salivary cotinine levels were not significantly correlated with probing depth and attachment loss. They explained that this phenomenon might, at least in part, be a result of extensive local factors, plaque and calculus present in the Chinese population evaluated in their study. However, these previous investigations did not examine the effect of passive smoking on periodontal disease.

Arbes et al. (2001) showed that among persons in the United States who had never used tobacco, those exposed to passive smoke were more likely to display periodontal disease than were those not exposed to passive smoke. However, they examined ETS exposure solely on the basis of self-reported behaviour; furthermore, they did not adjust exposure to ETS as a periodontitis prevalence by other lifestyle factors including alcohol consumption. In terms of passive smoking defined as salivary cotinine levels of 1–7 ng/ml, passive smokers exhibited significantly higher numbers of teeth characterized by CAL ⩾3.5 mm than did non-smokers in this investigation. Moreover, multiple logistic regression analysis of passive smokers revealed an odds ratio of 2.87 (95% CI: 1.05–7.82) following adjustment for other lifestyle factors. Aligne et al. (2003) detected a dose–response relationship between children's cotinine levels and the likelihood of caries in deciduous teeth after controlling for numerous potential confounders; additionally, they noted that their study possessed advantages afforded by utilizing cotinine level, rather than subjective parental reports, to estimate ETS exposure. A dose–response relationship between a salivary cotinine level of 0–7 ng/ml and numbers of teeth with PPD⩾3.5 mm or with CAL⩾3.5 mm was analysed in the present investigation; however, no meaningful correlation was observed (data not shown). This phenomenon may be attributable to the limited number of subjects.

The most important limitation of the present study corresponded to its cross-sectional design. Information pertaining to periodontal disease, self-reported smoking status and salivary cotinine level was collected simultaneously. In addition, the passive smokers category consisted of both never and former smokers. However, the rate of former smokers among passive smokers was 30.5%, which was quite similar to that in non-smokers (26.4%). Furthermore, this investigation included 111 never smokers and 50 former smokers demonstrating salivary cotinine levels of 3 and 5 ng/ml, respectively. No difference in cotinine levels was detected between never and former smokers. Moreover, no significant difference in numbers of teeth with PPD⩾3.5 mm and with CAL⩾3.5 mm was evident between these two groups. However, the mean numbers of daily cigarettes and the duration were 19.7 and 14.9 years, respectively, among former smokers in the current study (data not shown). Former smoking exposure may affect the results regarding the effect of passive smoking on periodontitis.

Despite these constraints, this investigation displayed considerable strength, including smoking status estimated by cotinine level, which was adjusted by other confounding lifestyle factors. Longitudinal studies involving large populations are necessary as they could provide stronger evidence in terms of a causal role of smoking with respect to periodontitis.

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Address:
Satoshi Shizukuishi
Department of Preventive Dentistry
Osaka University Graduate School of Dentistry
1-8, Yamadaoka, Suita, Osaka 565-0871 Japan.
E-mail: shizuku@dent.osaka-u.ac.jp

Clinical Relevance

Risks of smoking with respect to periodontitis have been examined primarily in active smokers; however, little regarding periodontitis risk associated with passive smoke exposure appears in the literature. In the present study, multiple logistic regression analysis revealed that odds ratios for periodontitis in passive smokers relative to non-smokers classified in terms of salivary cotinine level were 2.87 (95% CI: 1.05–7.82) following adjustment for other lifestyle factors. These findings should motivate dentists and dental hygienists pertaining to promotion of tobacco cessation in their practices. In addition, a smoke-free environment should be provided in the workplace and at home for periodontal health.