**Table 1.**

## Clinical Parameters

| | Total (N=372) | | Male (N=290) | | Female (N=82) | |
|---|---|---|---|---|---|---|
| Parameter | Median | IQR* | Median | IQR | Median | IQR |
| Age (years) | 42.0 | 18.0 | 41.0 | 16.0 | 45.0 | 26.0 |
| %PD >35 mm | 25.0 | 42.1 | 29.6 | 42.0 | 17.9 | 31.9 |
| BMI kg/m$^2$ | 22.4 | 4.0 | 22.6 | 3.9 | 21.1 | 4.2 |
| Pack-years | 0.0 | 15.0 | 4.0 | 19.0 | 0.0 | 0.0 |
| Alcohol consumption (g/day) | 22.0 | 15.4 | 22.0 | 22.0 | 0.0 | 22.0 |
| Frequency of toothbrushing (times/day) | 2 | 1 | 2 | 1 | 2 | 1 |

* Interquartile range.

**Table 2.**

## Percentage and Cumulative Percentage of %PD ≥3.5 mm

| %PD | N | Cumulative % |
|---|---|---|
| 0.0 | 32 | 8.6 |
| 0.1-10.0 | 60 | 24.7 |
| 10.1-20.0 | 60 | 40.9 |
| 20.1-30.0 | 56 | 55.9 |
| 30.1-40.0 | 39 | 66.4 |
| 40.1-50.0 | 23 | 72.6 |
| 50.1-60.0 | 29 | 80.4 |
| 60.1-70.0 | 19 | 85.5 |
| 70.1-80.0 | 19 | 90.6 |
| 80.1-90.0 | 19 | 95.7 |
| 90.1-100.0 | 16 | 100.0 |
| Total | 372 | |

and the upper 20th percentile was 60.0%. Simple logistic analysis revealed that factors such as age, gender, alcohol consumption, smoking status, BMI, and frequency of toothbrushing were significantly associated with periodontitis ($P$ <0.05, Table 3).

**Table 3.**

## Association Between Periodontal Status and Variables

| Independent variables | % Subjects in Upper 20th Percentile of %PD ≥3.5 mm | | Odds Ratio (95%CI) |
|---|---|---|---|
| Age (years) | | | |
| 20-39 | 11.1 | * | 1.0 |
| 40-59 | 26.3 | * | 2.85 (1.59-5.11) |
| Gender | | | |
| Female | 9.0 | * | 1.0 |
| Male | 22.3 | * | 2.92 (1.28-6.66) |
| BMI | | | |
| <25.0 kg/m$^2$ | 15.1 | * | 1.0 |
| ≥25.0 kg/m$^2$ | 36.0 | * | 3.17 (1.79-5.61) |
| Alcohol consumption | | | |
| <33.0 g/day | 15.6 | * | 1.0 |
| ≥33.0 g/day | 34.7 | * | 2.87 (1.61-5.13) |
| Smoking status | | | |
| <15.0 pack-years | 13.5 | * | 1.0 |
| ≥15.0 pack-years | 36.2 | * | 3.62 (2.09-6.26) |
| Frequency of toothbrushing | | | |
| ≥2 times/day | 15.7 | * | 1.0 |
| ≤1 time/day | 25.0 | * | 1.78 (1.06-3.02) |

* $P$ <0.05.

When these variables were tested by CART analysis (Fig. 1), the factor demonstrating the greatest impact on %PD was pack-years of smoking. Nodes 2 and 3 included those subjects displaying ≤7.8 pack-years (PY) and >7.8 pack-years, respectively. Node 2 was partitioned into nodes 4 and 5, based on BMI (4 = ≤25.1; 5 = >25.1). Node 5 could not be subdivided into two significantly distinct groups for any categories; consequently, it was a terminal node. Node 4 was divided by age into node 8 (≤31.5 years) and node 9 (>31.5 years). Nodes 8 and 9 were both terminal nodes. Node 3 was partitioned into nodes 6 and 7: node 7 contained subjects characterized by >26.3 pack-years and was a terminal node. Node 6 was subdivided by BMI into node 10 (BMI ≤25.5) and node 11, (BMI >25.5). These results suggested that pack-years of smoking exerted the greatest impact on periodontitis risk, followed by BMI. Cut-off points for BMI in this study were 25.1 or 25.5, which were nearly identical to those values of being overweight as defined by WHO.[20] Node 7 was not influenced by any other factor, except smoking. Factors such as gender, alcohol consumption, and frequency of toothbrushing were not significant variables in this analysis.

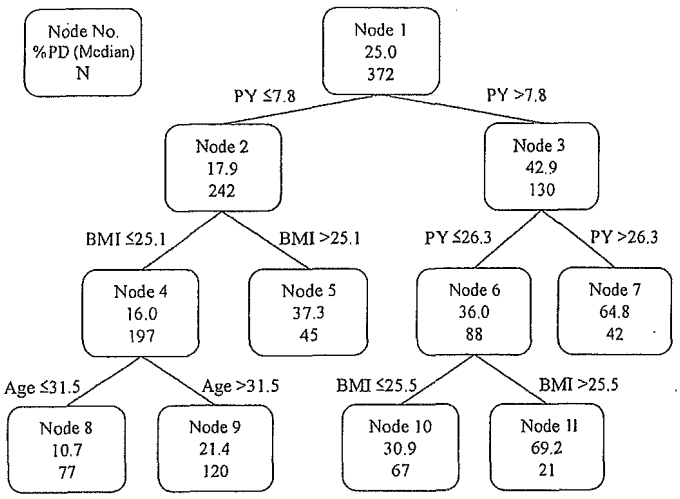


***Figure 1.***
*Classification and regression tree (CART) methods for evaluation of periodontal status. The first divided factor (the most important factor) was pack-years, followed by BMI and age. Abbreviations: PY; pack-years, BMI; Body Mass Index.*

**Table 4.**

## Risk for Periodontitis by Smoking Status

| | Pack-Years | | | | | *P* Value for Trend§ |
|---|---|---|---|---|---|---|
| | 0.0 | 0.1-9.9 | 10.0-19.9 | 20.0-29.9 | ≥30.0 | |
| N | 206 | 45 | 48 | 36 | 37 | |
| Age, years, mean | 40.9 | 30.6 | 37.8 | 45.0 | 49.6 | |
| %PD ≥3.5 mm, median | 18.2 | 18.2 | 32.7 | 42.9 | 60.7 | |
| Pack-years, mean | 0.0 | 4.8 | 14.5 | 23.6 | 44.4 | |
| Odds ratio* | 1.0 | 0.95 | 2.35 | 3.23 | 7.04 | <0.0001 |
| 95% CI | | 0.34, 2.65 | 1.08, 5.10 | 1.41, 7.38 | 3.19, 15.55 | |
| Odds ratio† | 1.0 | 1.32 | 2.68 | 2.70 | 5.27 | <0.0001 |
| 95% CI | | 0.45, 3.88 | 1.21, 5.94 | 1.16, 6.28 | 2.32, 11.98 | |
| Odds ratio‡ | 1.0 | 1.15 | 2.00 | 2.27 | 3.96 | 0.0017 |
| 95% CI | | 0.38, 3.52 | 0.86, 4.66 | 0.92, 5.63 | 1.59, 9.82 | |

* Unadjusted.
† Adjusted for age.
‡ Adjusted for age, gender, BMI, alcohol consumption, and frequency of toothbrushing.
§ Calculated across increasing categories of smoking for current smokers only. The linear trends in risks were evaluated using the average value for each category of smoking status.

In order to assess exposure to cigarette smoking on periodontitis, subjects were categorized into five groups according to their pack-years (Table 4). Never or former smokers were categorized as 0.0 pack-years and current smokers were categorized into four groups. Following adjustment for age, the odds ratio were 1.32 (95% CI: 0.45 to 3.88) for 0.1-9.9 pack-years, 2.68 (95% CI: 1.21 to 5.94) for 10.0-19.9 pack-years, 2.70 (95% CI: 1.16 to 6.28) for 20.0-29.9 pack-years and 5.27 (95% CI: 2.32 to 11.98) for ≥30.0 pack-years (*P* for trend <0.0001) as compared with 0.0 pack-years. %PD increased with increasing pack-years in a dose-dependent manner.

Subjects were subdivided into six groups according to BMI to evaluate obesity (Table 5). Following adjustment for age, the odds ratios were 1.87 (95% CI: 0.68 to 5.15) for BMI of 20.0-21.9, 1.88 (95% CI: 0.68 to 5.18) for BMI of 22.0-23.9, 2.84 (95% CI: 0.99 to 8.16) for BMI of 24.0-25.9, 4.55 (95% CI: 1.48 to 14.03) for BMI of 26.0-27.9 and 4.57 (95% CI: 1.30 to 16.06) for BMI ≥28.0 (*P* for trend = 0.0018) as compared with BMI <20.0. Further adjustments slightly attenuated these correlations. Increasingly poor periodontal status corresponded to increased BMI in a dose-dependent manner.

## DISCUSSION

The current study revealed a meaningful correlation between periodontitis and age, gender, BMI, smoking status, alcohol consumption, and frequency of toothbrushing via utility of simple logistic regression analyses. Furthermore, significant variables exhibiting greater risk for periodontitis were analyzed with the CART method. CART analysis affords numerous advantages;[17] CART results show independent variables in order of their impact on the dependent variable. The CART method also demonstrates the tendency with respect to earlier division (higher in the tree) as it relates to greater effect on severity of disease. Moreover, this approach stratifies for each variable and defines the cut-off points automatically. The most advantageous aspect of CART pertains to the graphical presentation of the results in an understandable manner despite their complexity.

The most potent factor identified by this investigation was smoking, followed by BMI and age (Fig. 1). Node 3 (Fig. 5) included those subjects who smoked more than 7.8 pack-years, considered as light smokers, suggesting that even light smoking status may affect periodontitis. Node 7 included those subjects who smoked in excess of 26.3 pack-years, heavy smokers characterized by high periodontitis risk. Node 11 subjects, who were moderate smokers displaying BMI >25.5, also exhibited great risk of periodontitis.

**Table 5.**

## Risk for Periodontitis by Obesity

| | Body Mass Index (kg/m$^2$) | | | | | | *P* Value for Trend§ |
|---|---|---|---|---|---|---|---|
| | <20.0 | 20.0-21.9 | 22.0-23.9 | 24.0-25.9 | 26.0-27.9 | ≥28.0 | |
| N | 73 | 96 | 91 | 57 | 34 | 21 | |
| Age, years, mean | 35.3 | 41.6 | 41.5 | 42.7 | 41.8 | 40.9 | |
| %PD ≥3.5 mm, median | 14.3 | 22.0 | 25.0 | 35.7 | 42.9 | 39.3 | |
| BMI kg/m$^2$, mean | 18.8 | 21.1 | 22.9 | 24.9 | 26.9 | 30.5 | |
| Odds ratio* | 1.0 | 2.25 | 2.34 | 3.64 | 5.33 | 5.33 | 0.0006 |
| 95% CI | | 0.83, 6.08 | 0.86, 6.33 | 1.30, 10.24 | 1.76, 16.13 | 1.66, 18.33 | |
| Odds ratio† | 1.0 | 1.87 | 1.88 | 2.84 | 4.55 | 4.57 | 0.0018 |
| 95% CI | | 0.68, 5.15 | 0.68, 5.18 | 0.99, 8.16 | 1.48, 14.03 | 1.30, 16.06 | |
| Odds ratio‡ | 1.0 | 1.88 | 1.74 | 2.68 | 3.89 | 4.40 | 0.0057 |
| 95% CI | | 0.66, 5.37 | 0.61, 4.97 | 0.90, 7.98 | 1.20, 12.56 | 1.18, 16.44 | |

* Unadjusted.
† Adjusted for age.
‡ Adjusted for age, gender, pack-years, alcohol consumption, and frequency of toothbrushing.
§ Calculated across increasing categories of smoking for current smokers only. The linear trends in risks were evaluated using the average value for each category of BMI.

However, alcohol consumption did not demonstrate a significant effect on periodontitis according to CART, despite the significance determined by bivariate analysis. In persons of Asian extraction, alcohol sensitivity differs between individuals; in particular, this phenomenon depends on $ALDH_2$ genotypes.[21] We previously reported that alcohol consumption and $ALDH_2$ genotypes are important factors with respect to periodontal risk.[15] Thus, it may be necessary to add the factor of $ALDH_2$ genotypes to risk models in future studies in this population.

Age, smoking, and BMI displayed significantly independent effects on periodontal status in CART; consequently, we examined the dose-response relationship between severity of periodontitis and smoking or BMI in detail via adjustment for confounding factors including age. With regard to smoking status, subjects were classified into five groups according to their pack-years with former or never smokers categorized into 0.0 pack-years. Adjusted odds ratio of periodontitis, which increased with increasing pack-years, exhibited a dose-response association. This trend was significant despite adjustment for confounding factors such as age. Several reports pertaining to the dose-response relationship between smoking and periodontitis have been published.[3,22-25] We have also shown previously the dose-dependent manner between smoking and periodontitis; cigarettes/day represented smoking status and modified CPI score represented periodontal status.[14] Grossi et al. demonstrated the dose response between pack-years of smoking and periodontitis employing attachment loss[22] or bone loss[24] despite adjustment for confounding factors including age. The odds of more severe attachment and alveolar bone loss were 4.75 and 7.28 in heavy smokers (pack-years of 31.1 to 150.0), respectively. These data, which support our findings, suggest that smoking exerts cumulative detrimental effects on periodontal health.

When subjects were initially dichotomized into two groups according to BMI above or below 25.0 for bivariate analysis, the odds ratio of obesity for periodontitis risk was 3.17. When subjects were classified into six BMI categories, the odds ratio was less than 3.0 for BMI of less than 26.0; however, the odds ratio rapidly increased with a BMI of ≥26.0. This result is consistent with the cut-off points established by CART analysis. Wood et al. also documented the dose-response relationship between BMI and periodontitis, which was defined based on the presence of one or more periodontal sites characterized by both attachment loss of ≥3 mm and PD of ≥4 mm; however, the adjusted odds ratio for BMI ≥30 was 1.37.[12] Saito et al.[26] showed the dose response between periodontitis and obesity; subjects with BMI of 25.0 to 29.9 and >30.0 exhibited crude odds ratios of 3.4 and 8.6, respectively, based on PD ≥4.0 mm. Interestingly, in a later study, they noted that higher levels of BMI significantly increased the adjusted risk of periodontitis only in those subjects displaying high waist-hip ratio in comparison with subjects demonstrating low waist-hip ratio and the lowest category of BMI.[11] Thus, these findings, in concert with our data, suggest that obesity is associated with periodontitis risk.

Smoking and obesity are known to affect host immunity.[7,27,28] Both factors may also decrease blood flow in periodontal tissues of obese subjects, which promotes the development of periodontitis.[7,29] These immunological disorders or inflammation might be the reason that obese smokers tend to exhibit escalating poor periodontal status relative to non-obese and non-smoking individuals. Despite the primary limitation of

our findings obtained from a cross-sectional study, the results suggest that smoking exerts the strongest effect on periodontitis. Moreover, these data reveal that both smoking and obesity, which display a dose-response relationship with periodontitis severity, are potential risk indicators for periodontitis.

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**Association of Melanin Pigmentation in the Gingiva of Children With Parents Who Smoke**

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# Association of Melanin Pigmentation in the Gingiva of Children With Parents Who Smoke

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ABSTRACT. *Objective.* The association between gingival pigmentation and active smoking has been established. This investigation is the first to address the relationship between gingival pigmentation in children and passive smoking.

*Methods.* A case-control study was performed involving 59 nonsmoking children who were selected from patient records of a dental clinic in a rural town in Japan. The number of subjects was based on a power calculation. Two calibrated examiners independently observed labial gingiva via oral photographs.

*Results.* An interview determined that 61% of children had at least 1 smoking parent. Gingival pigmentation was observed in 71% to 78% of children. Interexaminer agreement was satisfactory ($\kappa = 0.73$). Percentage of smoking parents was higher in children with gingival pigmentation (70–71%) than in those who lacked pigmentation (35%). Odds ratios of parental smoking adjusted by age and gender were 5.6 (95% confidence interval: 1.5–20.0) and 5.4 (1.4–21.2) for the 2 examiners.

*Conclusion.* These findings suggest that excessive pigmentation in the gingiva of children is associated with passive smoking. The visible pigmentation effect in gingiva of children could be useful in terms of parental education. *Pediatrics* 2005;116:e186–e190. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2004-2628; *parental smoking, melanin pigmentation, gingiva, child.*

ABBREVIATIONS. ETS, environmental tobacco smoke; OR, odds ratio; CI, confidence interval.

Brownish or black pigmentation in human gingiva has been reported in several countries. The prevalence rate of gingival pigmentation is diverse according to race and country[1–9]; hence, heredity may be a background factor. Intake of antimalarial drugs including chloroquine[10] and quinidine[11] is also associated with oral pigmentation.

Pigmentation in human gingiva derives from melanin granules, which are synthesized in melanosomes of melanocytes.[12] Melanocytes were identified as dendritic cells at the basal layer of gingival epithelium. Melanosomes, which are transferred via dendritic processes to keratinocytes by phagocytic activity, are degraded as they ascend to the surface. Melanin is synthesized from tyrosine and dihydroxyphenylalanine via dopaquinone as a result of the oxidation activity of tyrosinase.[13]

Melanin pigmentation in gingiva is correlated with active smoking: smokers displayed a greater propensity toward pigmentation than did nonsmokers.[3–7,9] A dose-response relationship with prevalence was detected.[4–7] Prevalence of pigmentation decreased in relation to the number of years after smoking cessation.[14] These findings indicate a causal association between tobacco smoke and melanin pigmentation in gingiva. Gingival pigmentation often occurred in the labial area of anterior teeth.[3,6,7,9] Excessive pigmentation in palatal mucosa as a result of tobacco smoke is a rare phenomenon, except in instances of reverse smoking.[15]

The prevalence of gingival pigmentation in smokers increased and approached maximum levels on slight exposure to smoking in minimal categories of duration of smoking and number of cigarettes smoked.[5,7] This characteristic is indicative of the sensitivity of gingival melanocytes to tobacco smoke. The prevalence of gingival pigmentation of workers was compared according to smoking status in 2 workplace locations.[7] The prevalence of pigmentation for current, former, and never smokers was 81%, 27%, and 15%, respectively, among 163 factory workers, whereas those rates among 154 office workers were 85%, 70%, and 37%, respectively. The apparent distinction in the prevalence rates of nonsmoking workers may be attributable to differences in environmental tobacco smoke (ETS) between workplace locations.

To date, few investigations have addressed the association between oral disease and exposure to ETS. On the basis of analyses of data derived from the Third National Health and Nutrition Examination Survey in the United States, the odds ratio (OR) of ETS exposure exceeding 1 hour to periodontal disease was 1.57 (95% confidence interval [CI]: 1.15–2.16)[16]; moreover, that of children who were aged 4 to 11 years and displayed serum cotinine levels of 0.2 to 10 ng/mL to untreated pediatric caries was 2.1 (95% CI: 1.5–2.9).[17] These findings suggest effects of passive smoking on oral condition. Parental smoking status was examined to estimate correlation between

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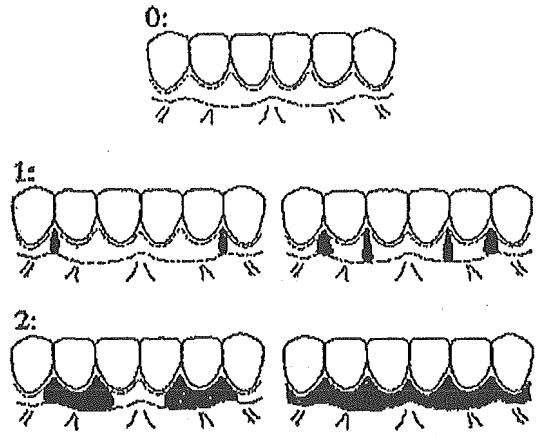


Fig 1. Classification according to extent of brownish or black pigmentation in labial gingiva of anterior teeth: 0, no pigmentation; 1, solitary unit(s) of pigmentation in papillary gingiva without extension between neighboring solitary units; and 2, formation of continuous ribbon extending from neighboring solitary units.

respiratory health and passive smoking in children.[18] The objective of the present study was to assess the relationship between melanin pigmentation in the gingiva of children and passive smoking.

## METHODS

### Determination of Sample Size

Preliminary documentation related to determination of sample size in the present case-control study is scarce. Furthermore, no published data pertaining to gingival pigmentation of children and parental smoking appear in the literature. Therefore, sample size was determined in accordance with the following reports: effect of active smoking on gingival pigmentation in adults[7] and the relationship between gingival pigmentation of children and their parents (Nakao Shimizu, DDS, written communication, 2002). Power analyses (Sample Power; SPSS Japan Inc, Tokyo, Japan) that were based on these reports indicated an appropriate sample size of 38 to 64 via consideration of overestimation with respect to the chance of exposure to tobacco smoke.

### Subjects and Smoking Status

Oral photographs of 59 children (22 boys and 37 girls) aged 6 to 16 years (11.3 ± 2.5) were selected randomly from patient records of a private dental clinic in Yamagata, which is located in the northern region of Japan. Informed consent was obtained; subsequently, smoking status of children and parents was established via interview. Images of the frontal mouth, which were acquired in a standardized manner, were evaluated for gingival pigmentation. Oral images were obtained with a digital camera (EOS D30; Canon Inc, Tokyo, Japan) equipped with wide-angle conversion lens (C-AF1 2X TELEPLUS MC7; Kenko Co, Tokyo, Japan). Parental smoking status was recorded separately on the basis of oral photographs.

### Evaluation of Melanin Pigmentation

Gingival pigmentation was assessed in the oral photographs, which were reproduced in a computer display. These reproductions exhibited size similar to that of the actual mouth. Brownish or black pigmentation in gingiva was classified according to extent of pigmentation unit in the labial aspect of anterior teeth (Fig 1). To date, no objective method for evaluation of gingival pigmentation has been developed. Gingival pigmentation was classified according to modification of melanin index categories[3]: 0, no pigmentation; 1, solitary unit(s) of pigmentation in papillary gingiva without formation of continuous ribbon between solitary units; and 2, at least 1 unit of formation of continuous ribbon extending from 2 neighboring solitary units. The current technique, which is subjective, was applied in children for the first time. Consequently, the reliability of this method was evaluated on the basis of interexaminer agreement: 2 examiners independently reviewed identical photographs. Examiners were trained and calibrated using typical photographs. Photographs in which visible pigmentation in the gingiva of children is apparent or lacking are presented in Fig 2. Gingival pigmentation of hemoglobin, melanoid, and carotene was obviously distinguishable from melanin pigmentation.[19] Several factors such as amalgam restoration adjacent to gingiva, melanoma, and long-term usage of antimalarial drugs[10,11] and minocycline[20] are potential confounders in terms of exposure to tobacco smoke; however, none of these parameters was applicable in this study. Status of parental smoking was withheld from the examiners. The current investigation was approved by the Ethics Committee of the Fukuoka Dental College.

### Statistical Analysis

Interexaminer agreement was evaluated for the existence and the extent of pigmentation with the $\kappa$ statistic. Additional analyses were performed using data sets that consisted of scores of gingival pigmentation assessed by the 2 examiners on the basis of parental smoking status, gender, and age. ORs of parental smoking with respect to gingival pigmentation were calculated via logistic regression analyses. Statistical analyses were conducted with software (SPSS; SPSS Japan Inc). The significance level was set at 5%.

## RESULTS

No child reported active smoking. At least 1 parent of 36 (61%) children smoked. These parents smoked for 19.8 (±4.2) years on average (±SD), consuming 19.3 (±9.0) cigarettes per day. Two thirds of these parents smoked >20 cigarettes per day. Distribution of gender and age was similar between children in both parental smoking groups (Table 1). $\kappa$ statistics for existence and extent of pigmentation were 0.73 and 0.68, respectively.

Gingival pigmentation was detected in 42 (71%) and 46 (78%) children by examiners A and B, respectively (Table 2). Prevalence of pigmentation was similar in boys and girls; 73% and 72% according to examiner A and 77% and 81% according to examiner B, respectively (data not shown). Solitary pigmentation was observed in 29% to 32% of the children. Continuous pigmentation, a form more distinct than solitary pigmentation, was noted in 42% to 46% of the children. Percentage of smoking parents was higher in children who displayed gingival pigmentation (70–71%) than in those who lacked pigmenta-

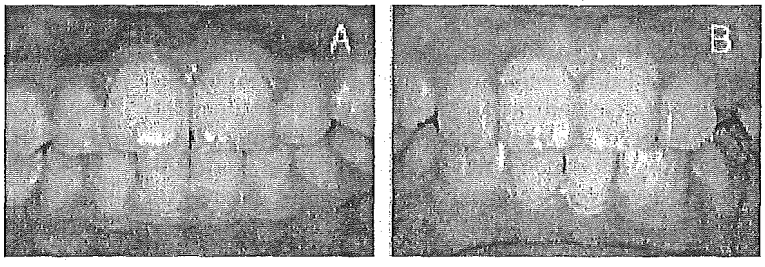


Fig 2. Typical photographs with (A) and without (B) visible pigmentation in the gingiva of children.

TABLE 1. Distribution of Gender and Age by Parental Smoking Status

| Smoking Status of Parents | Gender, *n* (%) | | Total | Age, Mean ± SD |
|---|---|---|---|---|
| | Male | Female | | |
| Nonsmoking | 9 (39) | 14 (61) | 23 | 11.4 ± 2.5 |
| Smoking | 13 (36) | 23 (64) | 36 | 11.3 ± 2.5 |
| Total | 22 (37) | 37 (63) | 59 | 11.3 ± 2.5 |

tion (35%). Percentage of smoking parents in children with solitary pigmentation (82%) was higher in comparison with those characterized by continuous pigmentation (64%).

Prevalence of gingival pigmentation in children with at least 1 smoking parent was 83% to 89%; in contrast, this condition was less prevalent (52–60%) in children whose parents did not smoke. Prevalence of gingival pigmentation in children was correlated significantly with smoking status of their parents (Table 3). Crude ORs of parental smoking were 4.6 (95% CI: 1.4–15.2, examiner A) and 5.1 (95% CI: 1.4–20.0, examiner B). Adjusted ORs relative to gender and age were 5.6 (95% CI: 1.5–20.0, examiner A) and 5.4 (95% CI: 1.4–21.2, examiner B).

## DISCUSSION

Examiners detected gingival pigmentation in 71% to 78% of children. Percentages of parental smoking on the basis of gingival pigmentation score and ORs were similar between examiners. Interexaminer agreement for existence and extent of gingival pigmentation was satisfactory as indicated by the $\kappa$ statistic. Thus, subjective evaluation of gingival pigmentation in children was sufficiently reliable so as to permit assessment of the association between gingival pigmentation in children and passive smoking.

Gingival pigmentation in Japanese children was described exclusively in an educational literature that introduced results of a survey regarding the prevalence among ~5000 children (age: 1–17 years) in metropolitan areas during 1982–1986.[21] Prevalence of gingival pigmentation increased and reached maximum levels in ~60% of children aged 1 to 6 years; moreover, prevalence continued at this level for nearly 7 years, followed by a gradual decrease to ~40% in children 17 years of age. No information related to smoking status was available; however, the value corresponding to prevalence of gingival pigmentation, which was smaller than that of the present investigation with respect to similar age group, may be attributable to differences associated with method of evaluation, eg, macroscopic versus photographic assessment. In adults, prevalence of melanin pigmentation was 15% to 37% for never smokers among Japanese workers.[7] Thus, prevalence in melanin pigmentation may differ between children and adults. Melanin pigmentation of oral mucosa was detected in 13.5% of Israeli children who were 6 to 10 years of age.[22] Melanin pigmentation varies in prevalence among different races and ethnic groups; for example, prevalence is higher in Asian populations (34.6%) in comparison with Ashkenazi (0.8%) and Sephardic (11.1%) Jews. In the present study, gingival pigmentation in the form of continuous ribbon was detected in 35% and 39% of children derived from the nonsmoking parent group. However, no comparable data exist regarding degree of pigmentation.

The significant association between parental smoking and gingival pigmentation in children suggests the presence of an ETS effect, which originated from parental smoking. No data corresponding to the exact amount of time that smoking parents and their children spent together were available. However, most parents smoked moderately or heavily; thus, their children may have been exposed to passive smoking for certain hours. In the 1999 report of the National Survey for Smoking and Health, 63.2% of Japanese were aware of the effect of ETS on asthma of children. Thus, a few parents in the smoking group may not smoke in the presence of their children. A second factor that skews the determination of the effect of ETS of parental origin is the impact of ETS from additional sources. ETS other than that of parental origin is likely to influence gingival pigmentation in the children. Passive smoking, other than that of parental origin, potentially may be similar or slightly higher in the smoking parental group than in the nonsmoking parental group. In both cases, the effect of ETS of parental origin on gingival pigmentation of children would be underestimated. National law restricting smoking in public areas went into effect in Japan on May 1, 2003.

Active smoking of participants was verified by interview. Parents or children who smoke might fail to report this activity. The smoking rate of male adults at ages corresponding to participating parents was ~60% in Japan. Because ~15% of women smoke, the smoking rate of parents (61%) was reasonable. In Japan, a national survey conducted in 1996 revealed that 0.7% of boys and 0.4% of girls at the age of ~13 years smoked. Therefore, we believe that the influence of smoking parents and children who failed to report is minimal. Future investigations that use determination of cotinine in urine[23] for validation of passive smoking in children could confirm direct association between passive smoking and gingival pigmentation. The present study suggested this relationship indirectly.

To date, a few reports appear in the literature regarding the potential sources that could stimulate melanin production in gingiva. An already high prevalence of gingival pigmentation was enhanced excessively by active smoking.[4] The stimulatory effect could be explained by high-affinity activity of polycyclic amines such as nicotine[24] and benzpyrene[25] in tobacco smoke on melanin; noxious substances in the epithelial cells were eliminated.

Two pathways by which stimulatory substances in ETS enter melanocytes in gingiva of children exist. One route involves penetration through oral mucosa; the second route is characterized by delivery via the bloodstream. Stimulatory agents of pigmentation in ETS can be introduced to saliva and might reach melanocytes through gingival epithelium. Gingival pigmentation was often observed in labial areas,[3,6,7,9] where ETS may not overlap directly. Furthermore,

TABLE 2. Distribution of Smoking Parents by Score of Melanin Pigmentation in the Gingiva of Their Children According to Observations of Two Examiners

| Examiner | Score of Pigmentation* | Smoking Status of Parents, *n* (%) | | Total |
|---|---|---|---|---|
| | | Nonsmoking | Smoking | |
| A | 0 | 11 (65) | 6 (35) | 17 |
| | 1 | 3 (18) | 14 (82) | 17 |
| | 2 | 9 (36) | 16 (64) | 25 |
| | 1 and 2 | 12 (29) | 30 (71) | 42 |
| B | 0 | 9 (65) | 4 (35) | 13 |
| | 1 | 6 (18) | 13 (82) | 19 |
| | 2 | 8 (36) | 19 (64) | 27 |
| | 1 and 2 | 14 (30) | 32 (70) | 46 |
| | Total | 23 (39) | 36 (61) | 59 |

* Scores of pigmentation: 0, no pigmentation; 1, solitary unit(s) of pigmentation in papillary gingiva without extension between neighboring solitary units; 2, formation of continuous ribbon extending from neighboring solitary units.

TABLE 3. OR and 95% CI of Gingival Pigmentation to Parental Smoking Status

| Examiner | Smoking Status of Parents | OR (95% CI) | |
|---|---|---|---|
| | | Crude | Adjusted* |
| A | Nonsmoking | 1.0 | 1.0 |
| | Smoking | 4.6† (1.4–15.2) | 5.6‡ (1.5–20.0) |
| B | Nonsmoking | 1.0 | 1.0 |
| | Smoking | 5.1§ (1.4–20.0) | 5.4‖ (1.4–21.2) |

* Based on multiple logistic-regression analysis controlling for gender and age.
† $P = .013$.
‡ $P = .010$.
§ $P = .016$.
‖ $P = .015$.

the majority of ETS is aspirated through the nose. Thus, indirect stimulation by nicotine and benzpyrene in ETS of gingival pigmentation via the bloodstream may afford a more plausible explanation.

The effect of parental smoking on gingival pigmentation in children was apparent; however, because the percentage of smoking parents of children who displayed solitary pigmentation was higher than that of children who presented with the more distinct form of continuous pigmentation, the effect in terms of extent of pigmentation was not clear. Additional studies using quantitative analyses with respect to effect of ETS and gingival pigmentation could establish greater detail regarding the association between melanin pigmentation in human gingiva and passive smoking.

Gingival pigmentation might be suggestive of parental smoking; however, gingival pigmentation was frequently observed in children, although prevalence of the symptom was higher in children with smoking parents in comparison with nonsmoking counterparts. Melanocytes normally occur in the gingiva of all humans.[19] Therefore, clinicians should not use gingival pigmentation as an indicator of parental smoking. The present investigation suggested an association between excessive pigmentation in the gingiva of children and passive smoking.

The impact of graphic warning labels of cigarette packages on adult smoking behavior was demonstrated in Canada[26]: two images depicting a diseased mouth and a lung tumor were identified as most effective at discouraging smoking; furthermore, the image of the mouth was selected by more smokers, female individuals, and young adults than its counterparts.[27] Gingival pigmentation in the mouth of a child is visible to parents and practitioners; as a result, melanin pigmentation in gingiva should be introduced to lists that pertain to children's health in relation to ETS,[28] which can be used by pediatric practitioners to educate parents with respect to the dangers of ETS.

## CONCLUSIONS

The oral diseases periodontitis and pediatric caries are related to passive smoking. The stimulatory effect of tobacco smoke on melanin pigmentation in gingiva was strong. This study is the first to describe the relationship between excessive pigmentation in the gingiva of children and parental smoking. This result is suggestive of the third effect of ETS exposure on oral symptoms: melanin pigmentation in gingiva of children. Future research may be necessary to confirm this finding such that the visible condition can be used in the education of parents.

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

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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 $\kappa$ 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.

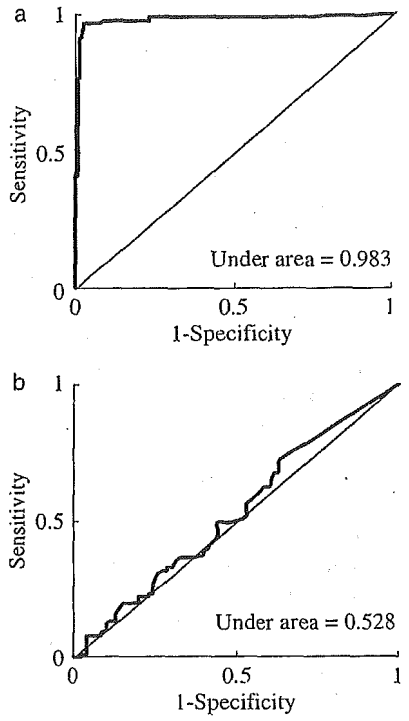


*Fig. 1.* ROC curves for assessment of salivary cotinine test ability in terms of detection of self-reported smoking status: (A) detection of current smoking status; (B) detection of ETS exposure status.

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 ⩽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 ⩾2.0 or ⩾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 ⩾ 3.5 mm | | | Number of teeth with CAL ⩾ 3.5 mm | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | mean | SE | mean rank | mean | SE | mean rank | mean | SE | mean rank |
| Active smokers (⩾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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**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.

# 歯科患者の喫煙への継続的介入に伴う禁煙ステージの移動

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浜島 信之[3*] 雫石 聡*

目的　歯科患者に継続的に簡便な喫煙の介入を行った場合の禁煙ステージ移動を調べ，介入による禁煙実行への実現可能性を検討した。
対象と方法　2001年4月から一定の条件で簡便な喫煙の介入を受け，2003年6-12月に禁煙ステージの評価を行った大学病院定期受診患者25人のステージ移動を後ろ向きに調査した。ステージの評価は，Prochaskaの分類に準じて行った。喫煙の介入は，患者自身の口腔に現れた喫煙関連疾患や症状および喫煙による治療効果の低下を受診機会毎に話題し，患者が禁煙の実行に関心を示した場合には禁煙方法を話題として禁煙希望に導いた。介入効果の評価は禁煙ステージの移動により検討した。
成績　対象者の定期受診間隔は1-6か月だった。介入前では，無関心期が15人，関心期5人，準備期が5人で，介入後は，それぞれ，6人，2人，1人であり，16人が禁煙を実行し，禁煙継続者は，9人だった。関心期，準備期には，それぞれ1人ずつが移動し，ステージ移動がみられたのは18人だった。ステージの移動がなかった7人のうち，無関心期のままは6人，関心期のままは1人であった。無関心期と関心期の20人のうち11人が，準備期の5人は全員が禁煙を実行した。
結論　継続的に簡便な喫煙の介入を行うことで歯科患者を禁煙の実行に誘導できることが示唆された。
Key words：歯科患者，喫煙介入，禁煙ステージの移動

## I 緒　　言

喫煙者が医療機関を受診する際は，健康上の不安が高まっていることから禁煙の助言を受け入れやすい。医科における介入は，一般外来[1]・禁煙外来[2]，入院[3]患者を対象とした報告がある。禁煙ステージ分類別の禁煙成功率では，禁煙準備期の者が最も高く，無関心期や関心期の喫煙者の禁煙成功率は低いが，医療機関を受診した喫煙者は，準備期の者は20%と少なく，無関心期や関心期の者が大多数であった[4]ことから，医療機関においては，禁煙準備に至らない者への介入も重要である。一方，患者の喫煙への介入の障壁として，患者の抵抗，時間がないなどがあげられている[5]。これらの点を考え，「禁煙しなさい」といった禁煙を強制する言葉を用いるなどの強い被指示性を伴わず，多数の喫煙者に簡便に提供できる禁煙誘導の方法が提案された[6]。

医療機関での介入における歯科固有の特徴は，米国で早くから認識されてきた。喫煙の口腔への悪影響は，口臭・歯の着色の身近な症状，歯周病や歯の喪失によるQOLの低下，生命の危険がある口腔がんなど健康への悪影響が多様であり，抜歯後治癒・歯周病治療・インプラントなど治療効果にも及ぶことから，喫煙の悪影響を，直接患者自身の身体で示して，様々な診療機会を通じて，あらゆる年齢層の患者に認識させることができるなど，歯科を受診する喫煙者は介入を受容しやすい状況にあることが指摘されている[7]。米国では，歯科患者への介入は早くから日常診療の一部となっている[8]。歯科診療所[9,10]および病院歯

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科[11]における介入効果が米国・英国で示されている。

喫煙者に対して医療機関や検診施設で，異なる分野の臨床専門家が，異なるタイミングと異なった形態で介入を行った場合，単一分野の臨床家だけが介入した場合と比べて，禁煙成功率が2.5倍以上高まる[12]。歯科は，医科と独立して機能する医療機関であることから，歯科患者への介入が普及すれば，医科と歯科の異なる分野の専門家から介入を受けることとなり，禁煙者が増加すると考えられる。そして，禁煙による効果が高いとされる20～45歳の者は，歯科を継続的に受診する[13]。歯科における介入の推進は，健康日本21の歯科保健，日本口腔衛生学会，日本公衆衛生学会禁煙宣言で示された。一方，歯学部同窓会員調査（1995年，545人，回収率70.6%）では，「患者の抵抗や不満」，「患者教育のための教材がない」，「時間がない」ことなどが介入を行う障壁と認識されていた[14]。歯科では，喫煙の健康への悪影響を喫煙者自身の口腔で確認できるので，日常診療の中で押し付け的な介入を行うことなく，患者を簡便に禁煙に誘導することができるのではないかと考えられる。本研究では，歯科患者に簡便な喫煙の介入(禁煙誘導)を行った場合のステージ移動を調べ，歯科における禁煙誘導実現の可能性について検討した。

## Ⅱ　研究方法

### 1.　対　象

大阪大学歯学部附属病院口腔保健科を2001年4月以降に受診し，1人の歯科医師から禁煙指導を受けた者を対象にし，患者の禁煙ステージが記録された既存の資料を用いて，介入（禁煙指導）前と介入後の禁煙ステージを比較して，介入効果を評価した。

なお，初診時の記録を基に介入前の禁煙ステージを判断し，介入後ステージに関しては2003年6月から12月の間を評価期間とした。この後ろ向き調査では25人が検討対象となり，年齢57±17歳，男18人，女7人であった。介入期間が6か月未満の者は3人であり，いずれも介入後のステージの評価期間中に介入を開始した者だった。この歯科医師の担当する患者の約10%が調査対象に該当した。分析にあたって個人情報は匿名化された。

### 2.　介入方法

喫煙への介入は，新しく開発された臨床歯科禁煙誘導法にしたがって，喫煙に関連する様々な口腔の話題（表1）を用いて禁煙に誘導した[7]。この方法では，禁煙への被指示性を少なくすることを意識して禁煙を直接の話題とせず，患者が禁煙に関心を示した場合に禁煙方法を話題として禁煙希望に導いた。本研究では，受診科の専門性から，歯周病治療および口腔清掃指導が誘導の主な

表1　歯科診療における禁煙誘導の機会と内容

| 機　会 | 内　　容 |
|---|---|
| 初診 | 喫煙・禁煙経験，歯の喪失，口腔癌，歯周病，口臭 |
| X線診査 | 妊娠と喫煙 |
| 歯科診査 | 歯：歯の喪失，歯（充填物）の着色 |
| | 歯周組織：歯根膜および歯槽骨へのニコチンの関与，セメント質へのニコチンの沈着，微小循環機能の異常，歯肉からの出血が少ない，歯肉の着色 |
| | 口腔粘膜：口腔癌，白板症，喫煙関連の口腔粘膜異常 |
| 歯周治療 | 歯石の増加，歯周治療（非外科的・外科的）の予後不良 |
| 保健指導 | 妊娠，歯石，歯（充填物）の着色，口腔癌，歯の喪失，歯周病の進行，歯肉の着色，若年者，女性，味覚異常，喫煙習慣，禁煙経験，喫煙者口唇，白板症，喫煙者口蓋 |
| 修復治療 | 歯の喪失，補綴物の予後，充填物の着色 |
| インプラント | インプラント失敗の可能性 |
| 抜歯 | 抜歯後の創傷治癒の遅延 |
| 定期健診 | 歯の喪失，歯周病の進行と再発，歯（充填物）や歯肉の着色，口臭 |
| 全ての診療の機会 | 喫煙・禁煙経験，全身の健康への影響，家族の健康への影響，受動喫煙，タバコ税・火災・未成年の喫煙など一般的な話，喫煙場所の制限 |

機会であった。対象者は，問診，歯科診査，治療時に歯科医師から，口腔清掃指導時に歯科衛生士各1人から介入を受けた。

3. 介入効果の評価

介入効果は，Prochaskaらの分類[15]を改変したステージ分類[16]を基準に，介入前後のステージ移動により評価した。介入前のステージは，初診時の問診票から，「あなたは禁煙について関心がありますか」という質問に対して，「禁煙のことをまだ考えていない」と答えた場合を無関心期，「禁煙に関心があるが，今すぐに（今後1か月以内に）実行しようとは思っていない」と答えた場合は関心期，「禁煙に関心があり，すぐにでも禁煙しようと思っている」と答えた場合は準備期とした。介入後のステージ分類では，介入評価期間に禁煙実行には至らなかった者には，無関心期，関心期，準備期のステージ分類を適用した。禁煙支援などにより禁煙を実行した場合のステージ分類は実行期とし，さらに，禁煙継続状況に関する質問の回答記録を調べ，禁煙継続状況が1年未満の者を短期成功とし，1年以上の者を長期成功とした。

## Ⅲ 研究結果

対象者の主な来院理由は，禁煙支援，歯周基本治療，支援的歯周治療（Supportive Periodontal Treatment, SPT），口臭治療であった。歯周基本治療では，患者は約1か月の間隔で，歯石除去・ルートプレーニングおよびブラッシング指導を3～4回受けた。SPTは，歯周基本治療により治癒した歯周組織の維持を患者本人に委ねるだけでなく，専門家が定期的に検査，口腔保健指導および専門的口腔清掃を行うもので，歯周病リスクの程度により3か月，6か月に1回の割合で受診した。口臭治療では，口臭検査，指導および専門的口腔清掃あるいは歯周基本検査を行い，患者は，約1か月に1回，通常2～3回受診し，その後定期健診を受けた。

調査対象者の介入前後の禁煙ステージ分布とステージ移動，禁煙実行別の喫煙者の属性および介入回数・期間を図に示した。介入前では，対象者25人中，無関心期が15人，関心期5人，準備期が5人であった。介入後には無関心期6人，関心期が2人，準備期が1人，禁煙実行者は16人であった。ステージ移動がみられたのは18人で，移動がなかったのは7人だった。介入前に無関心期であ

図　介入前後の禁煙ステージ分布とステージ移動，禁煙実行別の喫煙者の属性および介入回数・期間，禁煙実行者の禁煙継続状況