Association of second-hand smoke exposure, quantified by salivary cotinine, with dental caries in Japanese adolescents

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Original Article

Association of second-hand smoke exposure, quantified by salivary cotinine, with dental caries in Japanese adolescents

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Abstract

Purpose: Second-hand smoke has adverse effects on oral health. This cohort study used a multilevel approach to investigate the association of second-hand smoke exposure, as determined by salivary cotinine level, with dental caries in adolescents.
Methods: Data from 75 adolescents aged 11 or 12 years and 2,061 teeth without dental caries were analyzed in this study. Annual dental examinations to assess dental caries were conducted between 2018 and 2021. Salivary cotinine and Dentocult SM-Strip level were measured at baseline. Information on the smoking habits of parents, snack frequency, regular dental visits, and use of fluoride toothpaste was collected at baseline from parent-reported questionnaires.
Results: During the 3-year follow-up, dental caries was noted in 21 adolescents and 43 teeth. Participants exposed to parental smoking had higher salivary cotinine levels than those whose parents did not smoke. The multilevel Cox regression model showed that a high salivary cotinine level was associated with the incidence of dental caries, after adjusting for potential confounding factors (hazard ratio, 3.39; 95% confidence interval 1.08-10.69).
Conclusion: This study suggests that the risk of dental caries is higher for adolescents who have high salivary cotinine levels attributable to second-hand smoke exposure.

Keywords: cotinine, dental caries, second-hand smoke

Introduction

Second-hand smoke exposure causes multiple adverse health effects and is a global public health issue [1]. It is categorized as a human carcinogen (Group 1) by the International Agency for Research on Cancer (World Health Organization; 2007). Children are vulnerable to second-hand smoke exposure because they have limited control over their indoor environments [2]. Approximately 40% of children worldwide are exposed to second-hand smoke. In Japan, tobacco control remains insufficient [3]. Despite ratifying the World Health Organization's Framework Convention on Tobacco Control in 2004, it has lagged in tobacco control: a school-based nationwide survey found that 40% of Japanese aged 13-18 years were exposed to second-hand smoke [4].

Increasing epidemiological evidence suggests that second-hand smoke exposure is associated with dental caries in children and adolescents [5]. However, most of these studies had a cross-sectional design [5]. A recent meta-analysis found limited evidence for a causal relationship between second-hand smoke exposure and dental caries in children and adolescents [5], and four longitudinal studies reported an association with early childhood caries in primary teeth [6-9]. One cohort study in adolescents aged 13 years showed an association with dental caries in permanent teeth [10]. Additional evidence is required to confirm a longitudinal association in children and adolescents, especially for permanent teeth.

Second-hand smoke exposure in children and adolescents is often estimated using a parent-reported questionnaire. However, the collected data may be incorrectly interpreted because of recall and social desirability biases [11]. Biomarkers of nicotine intake, such as cotinine, are generally considered a more valid and reliable method for estimating second-hand smoke exposure [12]. Cotinine—the primary metabolite of nicotine—has a longer half-life than nicotine (16-20 h in children) [12], and several cross-sectional studies have shown that high cotinine levels are associated with dental caries in children [13,14] and adolescents [15]. However, it is unknown whether cotinine levels are longitudinally associated with dental caries in permanent teeth. To the authors' knowledge, no cohort study has examined this association.

This prospective cohort study investigated the longitudinal association between second-hand smoke exposure, as determined by salivary cotinine level, and caries in the permanent teeth of Japanese adolescents.

Materials and Methods

Study population

This study was conducted in an elementary and junior high school in the town of Hisayama, a suburb of Fukuoka—a metropolitan area in southern Japan. The inclusion criterion was enrollment in the sixth grade (age 11-12 years). The second molars of these children had begun to erupt and they had most of their permanent teeth. The exclusion criterion was absence of consent for participation. In April 2018, 94 sixth-grade students from all elementary schools in Hisayama were invited to participate in this study; 81 students provided written informed consent from their parents or guardians and were enrolled in the study. Dental examinations were performed annually between 2018 and 2021 as part of the Annual National Health Checkup. All examinations were performed in accordance with the School Health and Safety Act. The study protocol was approved by the Kyushu University Institutional Review Board for Clinical Research (Approval No. 21182-00).

Oral examination

The dental caries history of the participants' permanent teeth was assessed in accordance with the fifth edition of Oral Health Surveys: Basic Methods (World Health Organization; 2013). The permanent dentition status of each tooth was classified as a sound tooth with no evidence of treated or untreated caries, a tooth with untreated caries, a tooth filled because of caries, or a tooth missing because of caries. Decayed, missing, and filled teeth (DMFT) values were calculated. A permanent tooth was classified as having dental caries if it was decayed, filled, or missing.

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Assessment of second-hand smoke exposure

Second-hand smoke exposure was assessed by using salivary cotinine levels and parent-reported questionnaires. Saliva samples stimulated by chewing paraffin wax were collected at baseline and stored at −80°C until analysis. Salivary cotinine levels were measured with a commercially available quantitative enzyme immunoassay, in accordance with the manufacturer's protocol (Salimetrics, LLC., State College, PA, USA). The lower limit of sensitivity for the assay was 0.15 ng/mL. All values below this cutoff were recorded as 0.15 ng/mL.

The parent-reported questionnaire included two questions to assess subjective exposure to second-hand smoke. In the analysis of parental smoking, paternal and maternal smoking status (i.e., current, former, and never smokers) were assessed separately. The smoking status of fathers and mothers was categorized as current smoker or not. The participants were divided into three groups by combining paternal and maternal smoking status, as follows: neither parent smokes, one parent smokes, and both parents smoke. Assessment of household passive smoking was determined by the answer to the questions, "Do any family members smoke cigarettes?", and "If yes, does this member smoke in front of children?" The answers were used to divide participants into three groups: no family members smoke, family members do not smoke in front of children, and family member smokes in front of children.

Covariates

Information on participants' snack frequency, regular dental visits, and use of fluoride-containing toothpaste was obtained with a parent-reported questionnaire. Snack frequency was classified as ≤1, 2, and ≥3 times per day. Participants were categorized as those who did or did not regularly visit a dentist to prevent dental caries in the past year. Use of toothpaste containing fluoride was assessed with the question, "Do you use toothpaste containing fluoride?" The answer options were: "use toothpaste containing fluoride", "use toothpaste without fluoride", "use toothpaste but do not know if it contains fluoride", and "no use of toothpaste". The latter three options were combined to "no use of toothpaste containing fluoride".

A commercially available kit, Dentocult SM-Strip (Orion Diagnostica Co., Ltd., Espoo, Finland), was used to estimate levels of mutans streptococci. After saliva samples were collected, a Dentocult SM-Strip was rotated on the participant's tongue and gently withdrawn with the teeth apart and lips closed to obtain a thin layer of saliva. The growth density on the strips was classified according to the manufacturer's instructions and scored as 0, 1, 2, or 3, which corresponds to mutans streptococci growth levels of $<10^4$, $<10^5$, 10^5-10^6, and $\geq 10^6$ CFU/mL, respectively.

Statistical analysis

Quartiles of salivary cotinine levels were divided into two groups: the highest quartile (≥0.27 ng/mL) and three lower quartiles (<0.27 ng/mL). The chi-square test for categorical variables, Mann-Whitney *U* test for comparisons of continuous variables between two groups, and Kruskal-Wallis test for comparisons of continuous variables between three groups were used, with significance defined as $P < 0.05$ in two-tailed testing. Multiple comparisons between the three groups were performed with Bonferroni correction.

Data on dental caries have a natural hierarchical structure of teeth nested within individuals [16]. Each participant has data for multiple teeth. Tooth position may affect the pattern of caries development [16]. When analysis is performed at the individual level, tooth-specific information is lost. Therefore, a multilevel analytical framework—specifically, multilevel Cox proportional hazard regression analysis of time-to-event data—was adopted in this study. This model accounts for clustering of teeth within participants by treating the participant as a random effect. The dependent variable was occurrence of the event (incident dental caries) and the number of years from the baseline or examination year when the tooth erupted, to the event or censoring. The variables included in the multilevel Cox proportional hazard regression model were (1) individual-related factors such as second-hand smoke exposure (i.e., salivary cotinine levels, parental smoking, or household passive smoking), sex, snack frequency, regular dental visits, use of toothpaste containing fluoride, Dentocult SM-Strip result and (2) tooth-related factors such as tooth position (i.e., type of tooth and dental arch). Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. All statistical analyses were performed using STATA SE V.15 (Stata Corp., College Station, TX, USA).

Table 1 Salivary cotinine level (ng/mL) according to passive smoking exposure, as reported on a questionnaire administered to parents

	n	Geometric mean (95% CI)	*P* value
Parental smoking†			0.004
Neither parent smokes	43	0.17 (0.1-0.18)	
One parent smokes	28	0.26 (0.19-0.35)§	
Both parents smoke	7	0.28 (0.12-0.66)	
Household passive smoking‡			0.006
No family members smoke	43	0.17 (0.15-0.18)	
Family members do not smoke in front of children	17	0.22 (0.16-0.31)	
Family member smokes in front of children	20	0.29 (0.19-0.43)¶	

Kruskal-Wallis test. †Excluding respondents with missing values (*n* = 3). ‡Excluding respondents with missing values (*n* = 1). §Compared with participants with nonsmoking parents ($P < 0.01$). ¶Compared with participants with nonsmoking family members ($P < 0.01$). CI, confidence interval

Table 2 Participant characteristics in relation to salivary cotinine level at baseline

	Salivary cotinine low (<0.27 ng/mL) (*n* = 60)	Salivary cotinine high (≥0.27 ng/mL) (*n* = 21)	*P* value
Female participants	30 (50.0)	12 (57.1)	0.573
Parental smoking†			
Neither parent smokes	38 (65.5)	5 (25.0)	0.007
One parent smokes	16 (27.6)	12 (60.0)	
Both parents smoke	4 (6.9)	3 (15.0)	
Household passive smoking‡			
No family members smoke	38 (63.3)	5 (25.0)	0.009
Family members do not smoke in front of children	11 (18.3)	6 (30.0)	
Family member smokes in front of children	11 (18.3)	9 (45.0)	
Snack frequency (times per day)			
≤1	42 (70.0)	13 (61.9)	0.260
2	14 (23.3)	4 (19.0)	
≥3	4 (6.7)	4 (19.0)	
No regular dental visit	39 (65.0)	9 (42.9)	0.076
No use of toothpaste containing fluoride	27 (45.0)	9 (42.9)	0.865
Dentocult SM-Strip level ≥1	12 (20.0)	11 (52.4)	0.005
DMFT§	0.23 ± 0.56	0.24 ± 0.77	0.432

n (%), chi-square test. †Excluding respondents with missing values (*n* = 3). ‡Excluding respondents with missing values (*n* = 1). §Mean ± SD, Mann-Whitney *U* test. DMFT, decayed, missing, and filled teeth

Results

Eighty-one participants completed the baseline examination, including assessment of dental caries, salivary cotinine levels, and other related factors, and 75 were followed for 3 years. After excluding 19 teeth with previous dental caries at baseline, data from 2,061 permanent teeth in the 3-year follow-up period were analyzed.

The geometric mean for salivary cotinine level was 0.21 (95% CI 0.18-0.24) ng/mL. The percentages of participants with salivary cotinine levels of <0.15 ng/mL (the assay's lower limit of sensitivity) and ≥1.0 ng/mL were 64.2% and 6.2%, respectively. Salivary cotinine level was associated with subjective second-hand smoke exposure (Table 1). Participants whose fathers or mothers smoked had higher salivary cotinine levels than those whose parents had never smoked (Table 1). Salivary cotinine levels significantly differed between participants whose family members did and did not smoke in front of their children (Table 1). Moreover, more participants in the highest quartile of salivary cotinine levels had a Dentocult SM-Strip result of ≥1 (Table 2).

During the 3-year follow-up period, incident dental caries was noted in 21 participants and 43 teeth. The results of multilevel Cox proportional hazard regression analysis of the incidence of dental caries are shown in Tables 3 and 4. Regarding individual-related factors, a high salivary cotinine level was significantly associated with the incidence of dental caries (HR 3.39, 95% CI 1.08-10.69) (Table 3). However, parent-reported exposure to second-hand smoke from parental smoking or household passive smoking was not associated with dental caries (Table 4). These results were adjusted for sex, snack frequency, regular dental visits, use of toothpaste containing fluoride, Dentocult SM-Strip levels, and tooth position.

Table 3 Results of multilevel Cox proportional hazards regression model analysis of teeth with incident dental caries (75 participants and 2,061 teeth)

		Teeth-year	Incidence rate per 1,000 teeth-years (95% CI)	HR (95% CI)
Fixed effect				
Individual level				
Cotinine	low	4,574	5.47 (3.69-8.09)	1
	high	1,391	12.94 (8.15-20.54)	3.39 (1.08-10.69)
Sex	male	2,917	7.20 (4.69-11.04)	1
	female	3,048	7.22 (4.75-10.96)	0.95 (0.35-2.56)
Snack frequency (times per day)	≤1	4,238	6.84 (4.76-9.85)	1
	2	1,150	10.43 (5.93-18.37)	2.11 (0.63-7.03)
	≥3	577	3.47 (0.87-13.86)	0.17 (0.02-1.41)
Regular dental visit	yes	2,467	6.08 (3.67-10.09)	1
	no	3,498	8.00 (5.53-11.59)	1.02 (0.34-3.06)
Use of toothpaste containing fluoride	no	3,394	5.30 (3.34-8.42)	1
	yes	2,571	9.72 (6.57-14.39)	1.67 (0.62-4.51)
Dentocult SM-Strip level	0	4,297	4.42 (2.82-6.93)	1
	≥1	1,668	14.39 (9.64-21.47)	3.52 (1.16-10.67)
Tooth level				
Type of tooth	incisor/canine/premolar	4,366	1.14 (0.48-2.75)	1
	first molar	808	39.19 (27.56-55.73)	43.53 (16.76-113.04)
	second molar	791	8.66 (4.13-18.17)	7.59 (2.41-23.93)
Dental arch	maxillary teeth	2,995	4.34 (2.52-7.47)	1
	mandibular teeth	2,970	10.10 (7.06-14.47)	2.86 (1.48-5.53)
Random effect				
Individual-level variance (SE)				1.45 (0.86)

The multilevel Cox regression model included teeth with incident dental caries as the dependent variable and salivary cotinine level as the independent variable. HR was adjusted for sex, snack frequency, regular dental visits, use of toothpaste containing fluoride, Dentocult SM level, type of teeth, and dental arch (maxilla vs mandible). HR, hazard ratio; CI, confidence interval

Table 4 Association between subjective exposure to second-hand smoke and incident dental caries

	HR (95% CI)
Parental smoking	
Neither parent smokes	1
One parent smokes	1.69 (0.56-5.10)
Both parents smoke	2.56 (0.40-16.51)
Household passive smoking	
No family members smoke	1
Family members do not smoke in front of children	1.02 (0.29-3.55)
Family member smokes in front of children	1.73 (0.48-6.20)

The multilevel Cox regression model included teeth with incident dental caries as the dependent variable and parental smoking or household passive smoking as the independent variable. HR was adjusted for sex, snack frequency, regular dental visits, use of toothpaste containing fluoride, Dentocult SM level, type of teeth, and dental arch (maxilla vs mandible). HR, hazard ratio; CI, confidence interval

Discussion

Second-hand smoke exposure, as reflected by salivary cotinine level, was associated with the incidence of dental caries during a 3-year follow-up period. These findings are consistent with those of previous studies [15,17] of serum cotinine levels and dental caries and extend the results of prior studies by confirming a longitudinal association. Although cotinine level was a significant predictor of dental caries, the association between subjective exposure to second-hand smoke and dental caries was inconclusive. Subjective measurement based on questionnaires addressed to parents may have affected the results by introducing response bias. Because measurement of cotinine levels is an objective biomarker, it is more accurate than and superior to subjective assessments of second-hand smoke exposure [18].

The salivary cotinine levels observed in the present study were similar to those described in the National Health and Nutrition Examination Survey (NHANES) in the United States [19], which reported a geometric mean serum cotinine level of 0.15 ng/mL (95% CI 0.13-0.18 ng/mL) in never smokers aged 12-19 years [19]. Salivary cotinine levels are slightly higher than those in serum (ratio, 1.36) [20]. Salivary cotinine levels in adolescents in NHANES were estimated to be 0.20 ng/mL. Moreover, a serum cotinine level of 1.00 ng/mL (estimated equivalent salivary cotinine level, 1.36 ng/mL) in children or adolescents was classified as high exposure to second-hand smoke [17,19]. In the present study, four participants had salivary cotinine levels greater than 1.36 ng/mL, which suggests that second-hand smoke exposure is relatively low in this population.

The association between second-hand smoke exposure and dental caries is biologically plausible, as second-hand smoke may contribute to growth of cariogenic bacteria. An experimental study reported that tobacco components, such as nicotine, promoted attachment of *S. mutans* to the tooth surface [21]. In addition, serum vitamin C levels are frequently low in children exposed to parental smoking [22], which may promote *S. mutans* growth and biofilm formation [23]. Indeed, the present participants with high salivary cotinine levels had higher levels of *S. mutans*, as assessed by Dentocult SM-Strips (Table 2). Thus, although most participants did not have high cotinine levels, cotinine appears to have had effects on dental caries in this sample.

Cotinine levels are considered to reflect certain unmeasured factors related to dental caries, such as socioeconomic status. Unfortunately, data on socioeconomic status were not available for this study. A lower parental educational level was associated with higher smoking rates [24], and salivary cotinine levels in children have been reported to differ in relation to parental socioeconomic status [25]. In addition, a study in Japan reported that children of parents with lower education levels had higher caries treatment rates [26]. The relationship between socioeconomic status and oral health may be attributable in part to differences in oral health behaviors [27]. Although oral health behaviors such as snack frequency, regular dental visits, and use of fluoride-containing toothpaste were not associated with dental caries in this study, the possibility of residual confounding by socioeconomic status cannot be eliminated after adjustment for factors related to oral health behavior.

The present analysis of parental smoking status revealed that paternal and maternal smoking rates were similar to those reported in a Japanese national survey. In the present study 42.3% of fathers and 12.8% of mothers were current smokers; in the national survey, conducted in 2018, the rates were 37.0% for men and 13.6% for women aged 40-49 years (Japanese Ministry of Health Labour and Welfare; 2018). However, dental caries was less severe in the present sample than in the population described in the national survey. The DMFT value was 0.36 in the present second-year dental examination (i.e., first grade of junior high school) and 0.70 at the same age in the national survey of 2019 (Ministry of Education Culture Sports Science and Technology Japan; 2019), respectively. Hisayama conducted a school-based fluoride mouth-rinsing program in elementary school but not in junior high school. All participants received a fluoride mouth rinse once a week during elementary school. Additionally, participants received dental health guidance twice a year and brushed their teeth after lunch at school. The lower level of dental caries in this study may be the result of this school-based caries-prevention program. Nevertheless, despite the relatively better oral health of the children, second-hand smoke exposure was associated with dental caries.

In Japan, restrictions on public smoking have recently been implemented in accordance with the Health Promotion Law [28]. Many workplaces have introduced smoking policies, and smoking has been banned on public transportation in Japan. However, smoking is difficult to manage at home; thus, much of the public health burden from second-hand smoke falls on children who are exposed at home [29]. The present findings, as well as clear evidence of the harmful effects of parental smoking on respiratory diseases in children [30], confirm the need to increase public health awareness, so that parents can be induced to quit smoking, thus substantially benefiting the oral and systemic health of their children.

This study has several limitations. First, as mentioned above, information was not available on participants' socioeconomic status, such as household income and parental education level. Second, sugar consumption, which is related to dental caries, was not evaluated, although snack frequency was included in the analysis. Third, the present study did not investigate whether smoking parents used combustible cigarettes or heated tobacco. Heated tobacco is becoming increasingly popular among smokers in Japan [31], and evidence suggests that children are more likely to be exposed to second-hand smoke from heated tobacco than from combustion cigarettes [32]. Additionally, cotinine levels were lower among children exposed to second-hand smoke from heated tobacco than among those exposed to second-hand smoke from combustible cigarettes [32]. Some of the present participants whose parents smoked were presumed to have been exposed to second-hand smoke from heated tobacco, because most participants had low salivary cotinine levels. Future studies should examine the types of tobacco products and their effects on children. Fourth, this study did not evaluate behavioral change during the study period. However, among junior high school students in Japan, only 0.5% had smoked during the previous month (Cabinet Office, Government of Japan; 2018). Thus, it is unlikely that a substantial number of the present participants were smokers. Fifth, the severity of dental caries was lower in this study than in a Japanese national survey. Therefore, the present participants may not be representative of the general population. Finally, this study included elementary and junior high school students from a single municipality in Japan. The small sample size limits the ability to extrapolate the present findings to all Japanese adolescents. Caution is therefore warranted when generalizing the present findings to the Japanese population.

In conclusion, high salivary cotinine levels were longitudinally associated with dental caries in Japanese adolescents, which suggests that cotinine level can predict incident dental caries.

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Conflicts of interest
The authors have no conflicts of interest to declare.

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