

# Oral Hygiene and Risk of Nasopharyngeal Carcinoma—A Population-Based Case–Control Study in China

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## Abstract

**Background:** The association between oral health and risk of nasopharyngeal carcinoma (NPC) is largely unknown. Further understanding could shed light on potential pathogenic mechanisms and preventive measures.

**Methods:** We conducted a population-based case–control study in southern China between 2010 and 2014. We enrolled 2,528 incident NPC cases, aged 20–74 years, and 2,596 controls, randomly selected from the total population registers, with frequency matching to the 5-year age and sex distribution of the cases by geographic region. We interviewed subjects using a structured questionnaire inquiring about oral health indicators and potential confounding factors. We used unconditional logistic regression to estimate multivariate-adjusted ORs with 95% confidence intervals (CI).

**Results:** A higher number of filled teeth was associated with an elevated risk of NPC. Individuals with 1 to 3 and more

than 3 teeth filled versus none had adjusted ORs of 1.25 (95% CI, 1.06–1.49) and 1.55 (95% CI, 1.13–2.12), respectively ( $P_{\text{trend}} = 0.002$ ). Conversely, the adjusted OR for those who brushed teeth twice or more per day versus once or less per day was 0.62 (95% CI, 0.55–0.70). We detected a borderline significant positive association with earlier age at first adult tooth loss.

**Conclusion:** Our study suggested a positive association between some indicators of poor oral health and risk of NPC. Further studies are needed to confirm whether the findings are causal and, if so, to further explain the underlying mechanisms.

**Impact:** Improvement of oral hygiene might contribute to reducing NPC risk. *Cancer Epidemiol Biomarkers Prev*; 25(8); 1201–7. ©2016 AACR.

## Introduction

Epidemiologic studies suggest that poor oral health is associated with increased risks for cancers of the head and neck, esophagus, stomach, and pancreas (1–9). Although the underlying mechanisms are largely unknown, pathogenic shift in the oral microbiome could cause chronic inflammation in the aerodigestive tract (10–12), which in turn may influence cancer pathogenesis in this region (13). To our knowledge, few studies have investigated whether poor oral health affects nasopharyngeal

carcinoma (NPC) risk. Higher antigen levels of Epstein-Barr virus (EBV), a potential causative agent of NPC, were found in human lymphoblastoid P3HR-1 cells with culture fluid of *Fusobacterium nucleatum* (14). Poor oral health can increase the risk of NPC by stimulating EBV replication, as suggested by the finding of higher EBV load among individuals with periodontal disease than those without (15–18). Poor oral hygiene can also nurture oral bacterial overgrowth, and some oral bacteria may catalyze the production of nitrosamines, which are known carcinogens for NPC development (12, 19). Only one hospital-based case–control study in

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Turkey has addressed this research question and found a significant positive association between infrequent tooth brushing or higher number of decayed teeth and risk of NPC (20).

An unresolved question in the previous study is whether residual confounding by smoking, low socioeconomic status, and/or diet could explain associations with poor oral health. Detailed information on risk factors collected in a large, population-based study would help facilitate the rigorous evaluation of potential behavioral risk factors for NPC. To date, however, no such study of NPC has been conducted in southern China, where NPC is endemic, with incidence rates over 20 per 100,000 among men and 10 per 100,000 among women (21). Therefore, to better understand the causes and prevent this major regional public health problem, we investigated the association between oral health and risk of NPC in a population-based case-control study set in two southern Chinese provinces with the world's highest incidence rates of NPC (21–23).

## Materials and Methods

### Study population

The collaborative NPC Genes, Environment, and EBV study (NPCGEE) was conducted in the Zhaoqing area of Guangdong Province and the Wuzhou and Guiping/Pingnan areas of Guangxi Autonomous Region. Together, these three areas comprise 13 cities/counties in southern China, with a total population of approximately 8 million. Eligible cases were aged between 20 and 74 years at diagnosis, living in the described geographic area, and without a prior history of malignant disease or congenital or acquired immunodeficiency. To ensure prompt and nearly complete case ascertainment, we established a rapid case recruitment system including 10 hospitals and 2 cancer research institutions that directly notified study investigators of newly diagnosed NPC cases. In the Zhaoqing area, 1,528 eligible cases with NPC were identified between March 2010 and August 2013; in the Wuzhou area, 792 eligible cases with NPC were identified between April 2010 and September 2013; and in the Guiping/Pingnan area, 727 eligible cases with NPC were identified between July 2010 and December 2013. The number of cases identified in each region was close to the expected number of incident NPC cases on the basis of historical incidence rates. Of eligible patients who were contacted by study staff, 1306 (85% of 1528 cases) in the Zhaoqing area, 689 (87% of 792 cases) in the Wuzhou area, and 559 (77% of 727 cases) in the Guiping/Pingnan area were interviewed. All cases were histopathologically confirmed by pathological reports.

Computerized total population registers covering the Zhaoqing, Wuzhou, and Guiping/Pingnan populations are continuously updated by the local Public Security Bureau. Controls were randomly selected every 6 to 12 months from total population registries, with frequency matching to the 5-year age and sex distribution of the cases by geographic region. Controls were residents of the study area without a prior history of malignant disease or congenital or acquired immunodeficiency. Potential controls with outdated contact information or a history of working outside of the study area for more than 10 years, as identified with the help of the local government in each town or community, were replaced. Between November 2010 and July 2014 in the Zhaoqing area, between September 2011 and November 2014 in the Wuzhou area, and between October 2011 and October 2014 in the Guiping/Pingnan area, we randomly selected 3,932 poten-

tial controls. Of these, 730 (19%) could not be identified. Of the 3,202 who were identified, 138 (4%) had emigrated out of the study area, 90 (3%) were deceased or incapacitated, and 326 (10%) refused to participate. Of the 2,648 (83% of 3,202) enrolled controls, 2,133 (81%) were initial selections from the population registry and the other 515 (19%) were replacements for noncontacted selections. By comparing the distributions of age and sex between participating and nonparticipating controls in the Zhaoqing area, we found that younger persons were more likely that older persons to refuse to participate but that participation rates were similar between males and females (data not shown).

### Data collection

An electronic structured questionnaire was used by trained interviewers to conduct face-to-face or audiotaped telephone interviews with study participants. To reduce interviewer bias, we required each interviewer to interview an approximately equal number of cases and controls; however, blinding to case-control status was not feasible. Collected information covered demographic characteristics, residential history, occupational history, history of chronic ear, nose, and respiratory tract conditions, family medical history, dietary habits, cigarette smoking, alcohol and tea drinking, and use of Chinese herbal medicine. Questionnaire data were automatically flagged for logic errors and missing values, and errors were corrected by making comparisons against audio recordings or by recontacting participants.

Questions pertaining to oral health and hygiene addressed number of teeth lost after age 20 years, use of fixed dentures, number of teeth filled, daily frequency of brushing teeth, discomfort with eating particular foods, and food avoidance due to tooth or gum problems. Number of missing teeth after 20 years of age was categorized into four groups: none, 1 to 3, 4 to 13, and  $\geq 14$ . Age at first tooth loss was categorized into five groups:  $\geq 50$ , 40 to 49, 30 to 39, 20 to 29 years, and unknown. Number of filled teeth due to decay was categorized into three groups: none, 1 to 3, and  $\geq 4$ . Daily frequency of brushing teeth was categorized into three groups:  $\leq$  once per day, 2 or 3 times per day, and irregular. Frequency of oral discomfort was categorized into four groups: never or hardly ever, occasionally, often, and unknown. Avoidance of food due to oral problems was categorized as ever versus never.

One case and 17 controls had misplaced data, and 6 controls were not between ages 20 and 74 years at time of interview. Data on oral health were missing for 3 cases and 10 controls, and the overall quality of the questionnaire data was evaluated by the interviewer as poor for 26 cases and 15 controls. After excluding these 78 subjects, 2,528 cases and 2,596 controls were included in the present analysis.

### Statistical analyses

We compared differences in demographics and other potential confounders between NPC cases and controls by using the  $\chi^2$  test for categorical variables and Student *t* test for continuous variables. Multivariate unconditional logistic regression models were used to estimate ORs and corresponding 95% confidence intervals (CI) for risk of NPC associated with oral health indicators. The minimally adjusted multivariable model included the frequency-matching variables of age (in 5-year groups), sex, and residential area (Zhaoqing, Wuzhou, or Guiping/Pingnan).

Other potential confounders were selected in two steps: some were considered on the basis of prior knowledge and others were included if they changed the minimally adjusted OR of NPC for one increase of teeth lost by more than 10%. The *a priori*-selected covariates were attained education level ( $\leq 6$ , 7–9, 10–12, or  $>12$  years), current housing type [building (concrete structure), cottage (clay brick structure), or boat], current occupation (unemployed, farmer, blue-collar, white-collar, or other/unknown), first-degree family history of NPC (yes, no, or unknown), cigarette smoking (ever or never), tea drinking (daily or less than daily), and salt-preserved fish consumption in 2000–2002 (approximately 10 years prior to the interview; yearly or less, monthly, or weekly or more). Ever smokers were defined as those who reported having smoked at least one cigarette every 1 to 3 days for 6 months. In alternative models, we also adjusted for smoking status (never, former, or current) and cumulative smoking (pack-years). Former smokers were defined as those who had stopped smoking 4 years before the study interview. Forward stepwise confounder selection, in which the effect of adding one confounder at a time was evaluated, was further applied. The following variables were checked for confounding with the criterion of more than 10% changes in OR estimates that was described above: alcohol intake (ever or never), a self-reported history of chronic rhinitis (yes or no), and herbal medicine intake ( $\leq$  yearly or  $\geq$  monthly). Additional confounding by fruit and vegetable intake (g/day) was evaluated among 2,444 cases and 2,530 controls with reasonable values of total energy intake per day (700–4,200 kcal per day for males; 500–3,500 kcal per day for females; ref. 24). We tested for linear trends with ordinal variables in the models, using the median value within each category.

To control for residual confounding by smoking, we performed a subgroup analysis focusing on tobacco abstainers. We also performed a secondary analysis restricted to ever smokers. We evaluated the presence of effect modification by sex, age group, education level, cigarette smoking status, and salt-preserved fish consumption. Likelihood ratio tests for interaction terms were used to compare logistic regression models with and without an interaction term between oral health indicators and each potential modifier.

Analyses were performed with SAS version 9.4 (SAS Institute). All statistical tests were 2-sided, and  $P < 0.05$  was considered statistically significant.

### Sensitivity analyses

To reduce the potential for reverse causation, that is, an impact of NPC on oral health status or reporting, we conducted a sensitivity analysis restricted to cases interviewed within 30 days of diagnosis (2,166 cases, 86% of 2,528). Sensitivity analyses excluding the 32 replacement controls in Wuzhou who participated in an NPC screening program did not yield meaningfully different results and are not presented here.

### Ethical considerations

The study was approved by the institutional review boards of Sun Yat-sen University Cancer Center, Institute for Viral Disease Control and Prevention of Chinese Center for Disease Control and Prevention, Guangxi Medical University and Harvard TH Chan School of Public Health, as well as the Regional Research Ethics Vetting Board in Stockholm, Sweden. All subjects granted written or oral informed consent to participate.

**Table 1.** Characteristics of NPC cases and controls

Characteristics	Cases	Controls	P
	(N = 2,528) n (%)	(N = 2,596) n (%)	
Residential area			0.28
Zhaoqing	1,283 (50.8)	1,321 (50.9)	
Wuzhou	688 (27.2)	664 (25.6)	
Guiping/Pingnan	557 (22.0)	611 (23.5)	
Sex			1.00
Male	1,858 (73.5)	1,908 (73.5)	
Female	670 (26.5)	688 (26.5)	
Age at diagnosis/referral, y			<0.001 <sup>a</sup>
Mean (SD)	48.6 (10.6)	49.8 (10.9)	
20–29	82 (3.3)	80 (3.1)	0.007
30–39	423 (16.7)	372 (14.3)	
40–49	910 (36.0)	890 (34.3)	
50–59	686 (27.1)	729 (28.1)	
60–74	427 (16.9)	525 (20.2)	
Education level, y			0.003
$\leq 6$	1,005 (39.8)	932 (35.9)	
7–9	1,013 (40.1)	1,040 (40.1)	
10–12	403 (15.9)	483 (18.6)	
$>12$	107 (4.2)	141 (5.4)	
Current housing type			<0.001
Building (concrete structure)	1,818 (71.9)	2,019 (77.8)	
Cottage (clay brick structure)	700 (27.7)	574 (22.1)	
Boat	10 (0.4)	2 (0.1)	
Missing		1	
Current occupation			<0.001
Unemployed	77 (3.1)	95 (3.7)	
Farmer	855 (33.8)	984 (37.9)	
Blue-collar	1,020 (40.4)	900 (34.7)	
White-collar	350 (13.8)	416 (16.0)	
Other/unknown	226 (8.9)	201 (7.7)	
First-degree family history of NPC			<0.001
No	2,204 (87.2)	2,482 (95.6)	
Yes	272 (10.8)	70 (2.7)	
Unknown	46 (1.8)	43 (1.7)	
Missing	6 (0.2)	1 (0.04)	
Cigarette smoking			0.07
Never	1,117 (44.2)	1,213 (46.7)	
Ever	1,410 (55.8)	1,381 (53.2)	
Missing	1 (0.04)	2 (0.1)	
Tea drinking			<0.001
Less than daily	1,614 (63.8)	1,512 (58.2)	
Daily	911 (36.1)	1,081 (41.6)	
Missing	3 (0.1)	3 (0.1)	
Salt-preserved fish consumption in 2000–2002			0.02
Yearly or less	1,853 (73.3)	1,901 (73.2)	
Monthly	484 (19.2)	542 (20.9)	
Weekly or more	188 (7.4)	149 (5.7)	
Missing	3 (0.1)	4 (0.2)	

<sup>a</sup>P value was determined by a 2-sided *t* test. Other P values were determined by a  $\chi^2$  test.

## Results

### Study population characteristics

Table 1 shows the distribution of demographic characteristics and other potential risk factors for NPC among the 2,528 cases and 2,596 controls. Cases were slightly younger than controls and were more likely to be less educated, live in cottages, have blue-collar jobs, have a first-degree family history of NPC, have ever smoked, and have consumed salt-preserved fish at least weekly (Table 1).

### Associations between oral health indicators and risk of NPC

Table 2 shows adjusted ORs for the associations between oral health indicators and risk of NPC. We found no significant

**Table 2.** ORs and 95% CIs of NPC associated with oral health in southern China (2010–2014)

Variable	Cases (N = 2,528) n (%)	Controls (N = 2,596) n (%)	Minimally adjusted OR (95% CI) <sup>a</sup>	Fully adjusted OR (95% CI) <sup>b</sup>
Number of teeth lost after 20 y of age				
None	1,282 (50.7)	1,268 (48.8)	1.0 (ref.)	1.0 (ref.)
1–3	701 (27.7)	739 (28.5)	0.98 (0.86–1.12)	0.97 (0.84–1.11)
4–13	417 (16.5)	453 (17.5)	1.01 (0.85–1.19)	0.99 (0.83–1.18)
≥14	128 (5.1)	136 (5.2)	1.10 (0.84–1.45)	1.00 (0.75–1.32)
<i>P</i> <sub>trend</sub>			0.46	0.98
Age at first tooth loss, y <sup>c</sup>				
≥50	202 (16.2)	306 (23.0)	1.0 (ref.)	1.0 (ref.)
40–49	285 (22.9)	352 (26.5)	1.12 (0.87–1.44)	1.18 (0.91–1.53)
30–39	304 (24.4)	323 (24.3)	1.26 (0.96–1.64)	1.23 (0.93–1.62)
20–29	297 (23.8)	289 (21.8)	<b>1.34 (1.00–1.79)</b>	1.33 (0.98–1.79)
Unknown	158 (12.7)	58 (4.4)	—	—
<i>P</i> <sub>trend</sub>			0.04	0.08
Denture wearing				
No	2,161 (85.5)	2,214 (85.3)	1.0 (ref.)	1.0 (ref.)
Yes	367 (14.5)	382 (14.7)	1.06 (0.90–1.24)	1.03 (0.87–1.21)
Number of filled teeth				
None	2,077 (82.2)	2,223 (85.6)	1.0 (ref.)	1.0 (ref.)
1–3	352 (13.9)	297 (11.4)	<b>1.25 (1.06–1.47)</b>	<b>1.25 (1.06–1.49)</b>
≥4	99 (3.9)	76 (2.9)	<b>1.39 (1.03–1.89)</b>	<b>1.55 (1.13–2.12)</b>
<i>P</i> <sub>trend</sub>			0.003	0.002
Frequency of brushing teeth, times/d				
≤1	1,696 (67.1)	1,456 (56.1)	1.0 (ref.)	1.0 (ref.)
≥2	824 (32.6)	1,133 (43.6)	<b>0.58 (0.52–0.66)</b>	<b>0.62 (0.55–0.70)</b>
Irregular	8 (0.3)	7 (0.3)	—	—
Frequency of oral discomfort while eating				
Never or hardly ever	1,678 (66.4)	1,786 (68.8)	1.0 (ref.)	1.0 (ref.)
Occasionally	613 (24.3)	624 (24.0)	1.07 (0.94–1.22)	1.08 (0.94–1.23)
Often	206 (8.1)	169 (6.5)	<b>1.37 (1.10–1.70)</b>	<b>1.27 (1.02–1.59)</b>
Unknown	31 (1.2)	17 (0.7)	—	—
Avoidance of some foods due to oral problems				
Never	1,778 (70.3)	1,794 (69.1)	1.0 (ref.)	1.0 (ref.)
Ever	750 (29.7)	802 (30.9)	0.98 (0.86–1.10)	0.97 (0.85–1.10)

<sup>a</sup>Adjusted for sex, age (5-year categories), and residential area (Zhaoqing, Wuzhou, or Guiping/Pingnan).

<sup>b</sup>Adjusted for sex, age, residential area, education level (≤6, 7–9, 10–12, or >12 years), current housing type (building, cottage, or boat), current occupation (unemployed, farmer, blue-collar, white-collar, or other/unknown), first-degree family history of NPC (yes, no, or unknown), cigarette smoking (ever or never), tea drinking (less than daily or daily), and salt-preserved fish consumption in 2000–2002 (yearly or less, monthly, or weekly or more). We excluded 18 subjects due to missing covariate values.

<sup>c</sup>Among 2,574 participants who experienced tooth loss after the age of 20 years.

association with higher number of teeth lost in adulthood in models adjusted for sex, age and residential area only or additionally adjusted for education level, current housing type, current occupation, cigarette smoking, tea drinking, and salt-preserved fish consumption in 2000–2002. In the fully adjusted model, compared with no teeth lost, the ORs (95% CIs) for 1 to 3, 4 to 13, and 14 or more teeth lost were 0.97 (0.84–1.11), 0.99 (0.83–1.18), and 1.00 (0.75–1.32), respectively (*P*<sub>trend</sub> = 0.98). Among 2,574 subjects who had experienced tooth loss after the age of 20 years, we found a borderline significant trend toward increasing risk of NPC with earlier age at first tooth loss (*P*<sub>trend</sub> = 0.08). The fully adjusted ORs (95% CIs) for those with first tooth loss at 40–49, 30–39, and 20–29 versus ≥50 years were 1.18 (0.91–1.53), 1.23 (0.93–1.62), and 1.33 (0.98–1.79), respectively. We did not detect an association between denture wearing and risk of NPC after full adjustment (OR = 1.03, 95% CI, 0.87–1.21). Compared with those who had not had any teeth filled, those with 1 to 3, or more than 3 teeth filled had significantly increased risks of NPC, with ORs of 1.25 (95% CI, 1.06–1.49) and 1.55 (95% CI, 1.13–2.12), respectively (*P*<sub>trend</sub> = 0.002).

Brushing teeth twice per day or more was significantly and inversely associated with risk of NPC, with a fully adjusted OR of 0.62 (95% CI, 0.55–0.70), compared with less frequent brushing (Table 2). The fully adjusted OR for often experiencing oral discomfort while eating was 1.27 (95% CI, 1.02–1.59), compared with never or hardly ever experiencing such discomfort. We did not detect an association between avoidance of certain foods because of oral problems and risk of NPC. Further adjustment for alcohol intake, a self-reported history of chronic rhinitis, herbal medicine intake, and intake of green leafy vegetables (g/d) and fruits (g/d) in 2000–2002 or detailed smoking history (i.e., never, former, or current plus pack-years) did not meaningfully affect the results (data not shown). Among never smokers, no significant association was detected between number of adult teeth lost or age at first tooth loss and risk of NPC (Table 3), whereas earlier age at first tooth loss was associated with an increased risk of NPC among ever smokers (Supplementary Table S1). Otherwise, the magnitude of the associations with other oral health variables among never smokers was similar to that in the overall study population.

**Table 3.** ORs and 95% CIs of NPC associated with oral health among never smokers in southern China (2010–2014)

Variable	Cases (N = 1,117) n (%)	Controls (N = 1,213) n (%)	Minimally adjusted OR (95% CI) <sup>a</sup>	Fully adjusted OR (95% CI) <sup>b</sup>
Number of teeth lost after the age of 20 y				
None	601 (53.8)	623 (51.4)	1.0 (ref.)	1.0 (ref.)
1–3	297 (26.6)	327 (27.0)	0.97 (0.79–1.18)	0.97 (0.79–1.19)
4–13	174 (15.6)	195 (16.1)	0.98 (0.76–1.27)	0.98 (0.76–1.28)
14+	45 (4.0)	68 (5.6)	0.76 (0.50–1.16)	0.71 (0.46–1.11)
<i>P</i> <sub>trend</sub>			0.24	0.16
Age at first tooth loss (years) <sup>c</sup>				
≥50	74 (14.3)	108 (18.3)	1.0 (ref.)	1.0 (ref.)
40–49	94 (18.2)	135 (22.9)	0.82 (0.54–1.25)	0.94 (0.61–1.46)
30–39	127 (24.6)	156 (26.4)	0.87 (0.56–1.35)	0.92 (0.58–1.45)
20–29	149 (28.9)	164 (27.8)	0.89 (0.56–1.40)	0.90 (0.56–1.45)
Unknown	72 (14.0)	27 (4.6)	—	—
<i>P</i> <sub>trend</sub>			0.82	0.69
Denture wearing				
No	949 (85.0)	1,031 (85.0)	1.0 (ref.)	1.0 (ref.)
Yes	168 (15.0)	182 (15.0)	1.06 (0.83–1.34)	1.06 (0.83–1.35)
Number of filled teeth				
None	889 (79.6)	1,011 (83.4)	1.0 (ref.)	1.0 (ref.)
1–3	170 (15.2)	158 (13.0)	1.21 (0.95–1.53)	<b>1.28 (1.00–1.64)</b>
≥4	58 (5.2)	44 (3.6)	1.43 (0.96–2.15)	<b>1.61 (1.06–2.44)</b>
<i>P</i> <sub>trend</sub>			0.06	0.02
Frequency of brushing teeth, times/d				
≤1	676 (60.5)	606 (50.0)	1.0 (ref.)	1.0 (ref.)
≥2	434 (38.9)	604 (49.8)	<b>0.58 (0.49–0.69)</b>	<b>0.63 (0.52–0.76)</b>
Irregular	7 (0.6)	3 (0.2)	—	—
Frequency of oral discomfort while eating				
Never or hardly ever	763 (68.3)	814 (67.1)	1.0 (ref.)	1.0 (ref.)
Occasionally	256 (22.9)	310 (15.6)	0.89 (0.73–1.08)	0.91 (0.75–1.12)
Often	88 (7.9)	82 (6.8)	1.18 (0.85–1.62)	1.15 (0.83–1.60)
Unknown	10 (0.9)	7 (0.6)	—	—
Avoidance of some foods due to oral problems				
Never	789 (70.6)	801 (66.0)	1.0 (ref.)	1.0 (ref.)
Ever	328 (29.4)	412 (34.0)	0.82 (0.68–0.98)	0.83 (0.69–1.00)

<sup>a</sup>Adjusted for sex, age (5-year categories), and residential area (Zhaoqing, Wuzhou, or Guiping/Pingnan).

<sup>b</sup>Adjusted for sex, age, residential area, education level (≤6, 7–9, 10–12, or >12 years), current housing type (building, cottage, or boat), current occupation (unemployed, farmer, blue-collar, white-collar, or other/unknown), first-degree family history of NPC (yes, no, or unknown), tea drinking (less than daily or daily), and salt-preserved fish consumption in 2000–2002 (yearly or less, monthly, or weekly or more). We excluded 7 subjects due to missing covariate values.

<sup>c</sup>Among 2,358 participants who experienced tooth loss after the age of 20 years.

We found no statistically significant heterogeneity in the association with number of teeth lost by sex, age group, education level, first-degree family history of NPC, or salt-preserved fish consumption (Supplementary Table S2). We also observed no significant heterogeneity in associations with daily frequency of tooth brushing by potential effect modifiers (Supplementary Table S3).

In sensitivity analyses restricted to cases interviewed within 30 days of diagnosis, we did not detect any appreciable changes in the magnitude of associations (Supplementary Table S4). For example, compared with those who had not had any teeth filled, those with 1 to 3 or more than 3 teeth filled had significantly increased risks of NPC, with ORs of 1.34 (95% CI, 1.12–1.60) and 1.64 (95% CI, 1.18–2.26), respectively (*P*<sub>trend</sub> < 0.001). The fully adjusted OR for brushing teeth twice per day or more was 0.58 (95% CI, 0.51–0.66), compared with less frequent brushing.

## Discussion

In this large, population-based case-control study set in the NPC-endemic region of southern China, we did not find positive associations between higher number of teeth lost in adulthood or

denture wearing and risk of NPC. However, other indicators of poor oral health, such as a higher number of filled teeth, were significantly associated with an elevated risk of NPC, and associations with earlier age at first adult tooth loss, albeit statistically nonsignificant, were in the same direction. Conversely, brushing teeth twice per day or more was inversely associated with NPC risk. These findings were not confounded or modified by known risk factors for NPC.

To our knowledge, this is the first population-based case-control study to investigate the association between oral health and risk of NPC. Our results regarding number of filled teeth are somewhat in agreement with findings from a previous hospital-based case-control study in Turkey that found significant increased risks of NPC in association with infrequent tooth brushing (OR, 6.17; *P* < 0.001) and having more than 10 decayed teeth (OR, 2.17; *P* < 0.001; ref. 20). The stronger associations in the Turkish study may be due, in part, to poorer oral health and hygiene conditions in their study population than ours, although the differences may also be due to study design, confounding, bias, or chance.

Poor oral health and hygiene can lead to periodontal disease and caries, both of which are characterized by chronic bacterial infection (12, 19), the major cause of tooth damage and loss in

adults. Smoking, a weak-to-moderate risk factor for NPC, can also cause periodontal disease and tooth loss (25, 26) and may therefore confound the association between poor oral health and NPC. In the present study, we found no changes in the estimated associations between oral health and risk of NPC after adjusting for ever smoking or more detailed smoking status and pack-years. ORs for number of adult teeth lost and age at first tooth loss were lower than 1.0 among never smokers and above 1.0 among ever smokers, whereas the ORs for number of filled teeth were greater than 1.0 among never smokers. A significant inverse association with brushing teeth twice or more per day was detected among both never smokers and ever smokers. Thus, some associations may be affected by residual confounding by smoking history, although the effect should be minor.

We found a null association between number of adult teeth lost and risk of NPC; however, biologically plausible interpretations are possible. Number of adult teeth lost is a proxy for periodontal disease, which can lead to the release of inflammatory mediators that may play a role in the development of some types of cancer (10, 11). Although recall bias cannot be ruled out, the null association with number of teeth lost could be due to the younger ages of NPC onset (median age, ~45 years) than those of most of oral health-related cancers, such as cancers of the esophagus and pancreas (median age, ~70 years). In the present study, about 50% of the study population had not experienced any adult teeth lost; therefore, number of teeth lost may not be a sensitive indicator of oral health in this setting. Alternatively, the null association with number of teeth lost but the positive association with a higher number of filled teeth may suggest that etiologic mechanisms related to dental caries are more important in the development of NPC.

Currently, the underlying mechanistic pathways that may potentially link oral hygiene with risk of NPC are largely speculative. Individuals with poor oral health may have a higher proportion of oral bacterial flora that efficiently reduce nitrate to nitrite (27), a necessary step in the *in vivo* formation of carcinogenic nitrosamines (12, 28, 29). Recent studies have implicated EBV in the pathogenesis of periodontal disease (15, 16, 18, 30), with higher EBV DNA load in the saliva and periodontal pocket of patients with periodontal disease than those without (15, 31). Thus, it is conceivable that poor oral health could increase the risk of NPC development by facilitating EBV reactivation and replication, although poor oral health could also be a marker of EBV reactivation and replication.

Our results should be interpreted in light of several methodologic limitations. First, we lacked information on disease symptoms and stage, which may influence some oral health and hygiene measures (32). Second, selection bias favoring underestimated ORs could have occurred if, for example, lower socioeconomic status (SES) controls with poorer oral health and hygiene were more likely to participate than higher SES controls who could not be contacted because of employment outside of their hometown. Third, although all NPC cases were histopathologically confirmed, information on specific type was not available for some cases. Our study had a limited ability to detect heterogeneity among histopathologic types of NPC, and our results apply mainly to type II/III NPC, which comprises the majority (>95%) of NPC cases in southern China (33). Fourth,

information on receipt of professional dental care was not available, which precluded accounting for this potential confounder. However, in the study area, which is socioeconomically disadvantaged, the population seeking regular dental care is expected to be small. Given SES is positively associated with the probability of seeking regular dental care while negatively associated with NPC risk, our observed association between number of filled teeth and NPC risk might be even underestimated. Fifth, a slightly higher participation rate among older controls and delayed interview in some controls have resulted a slightly older control population, which may have led to an underestimated association between oral hygiene and the risk of NPC. Finally, all measures of oral health were self-reported and were not verified, for example, by direct examination of lost or filled teeth; therefore, misclassification could have resulted in either overestimated or underestimated associations. These limitations are offset by the study's strengths, which include its large size, population-based design, and setting in a relatively culturally and ethnically homogeneous NPC-endemic region.

In summary, we found that a higher number of filled teeth was significantly associated with an elevated risk of NPC. In addition, higher frequency of tooth brushing may be associated with a lower risk of NPC. Although other measures of oral health, particularly number of adult teeth lost, were not significantly associated with risk of NPC, our overall results suggest that poor oral health may increase risk of NPC. Prospective cohort studies are needed to rule out reverse causation, and biomarkers such as oral microflora profiles and EBV DNA load in saliva are needed to more deeply investigate the question of whether poor oral health is associated with the risk of NPC.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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