Oral Health and Risk of Upper Gastrointestinal Cancers in a Large Prospective Study from a High-risk Region: Golestan Cohort Study

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ABSTRACT

Tooth loss and periodontal disease have been associated with several cancers, and poor oral health may be an important risk factor for upper gastrointestinal (UGI, i.e., esophageal and gastric) cancers. We assessed the relationship between oral health and UGI cancers using a large prospective study of over 50,000 adults living in Golestan Province, Iran, a high-incidence area for these cancers. Hazard ratios (HRs) and 95% confidence intervals (CI) were estimated for the association between three different measures of oral health [frequency of tooth brushing; number of missing teeth; and the sum of decayed, missing, and filled teeth (DMFT)] and UGI cancers. During a median follow-up duration of 13 years, there were 794 incident UGI cancers (396 esophageal and 398 gastric cancers). Daily tooth brushing was associated with a decreased risk of developing both esophageal (HR = 0.670; 95% CI: 0.486–0.924) and gastric (HR = 0.741; 95% CI: 0.544–1.01) cancers (combined UGI cancer HR = 0.697; 95% CI: 0.558–0.871) compared with never brushing. Tooth loss in excess of the loess smoothed, age- and sex-specific median number of teeth lost was significantly associated with esophageal (HR = 1.64; 95% CI: 1.08–2.47) and gastric cancers (HR = 1.58; 95% CI: 1.05–2.38). There were some adverse associations between DMFT and UGI cancers but most were not statistically significant. These results suggest increased risk of developing UGI cancers among individuals with poor oral health, and those who do not perform regular oral hygiene.

Prevention Relevance: Poor oral health is associated with the risk of upper gastrointestinal cancers, and oral hygiene practices may help prevent these cancers.

Introduction

Cancers of the stomach and esophagus [i.e., upper gastrointestinal (UGI) cancers] are the third and sixth leading causes of cancer mortality in the world, respectively (1). The highest incidence rates of these cancers are commonly seen in Eastern and Central Asia, and both cancers demonstrate substantial geographic heterogeneity, where incidence can vary by 10-fold between high- and low-risk countries (1–3). In high-income countries such as the United States (4), tobacco smoking and alcohol intake are the primary risk factors for esophageal squamous cell carcinoma (ESCC), the predominant histologic type of esophageal cancer (2). However, these practices are uncommon in high-risk areas, including rural China (i.e., Linxian) and Iran, and parts of Africa, and contribute minimally to esophageal cancer in these populations (2, 5). Similarly, although smoking is an important risk factor for gastric cancer in high-income regions, it does not explain the high incidence in areas where smoking is uncommon (3, 6). Furthermore, *Helicobacter pylori* infection, an established risk factor for gastric cancer (3), was only associated with a relatively modest increase in risk in areas with high infection rates and high incidence of gastric cancer (7, 8). This suggests the

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etiology of UGI cancers are likely to involve other risk factors that have yet to be identified.

Poor oral health, indicated by periodontal disease and tooth loss, has been associated with an increased risk of various cancer types, including UGI cancers (9–14). Previous prospective studies conducted in rural China, where residents have poor access to dental care, have consistently shown an association between tooth loss and esophageal and gastric cancer incidence and mortality (15–17). An association between tooth loss and UGI cancers has been reported in other regions of the world, including Japan (18), parts of Europe (19–21), and Latin America (19), but there have also been inconsistent results in other areas (22–24). It is also unclear whether oral hygiene practices, such as tooth brushing, provide protective effects against UGI cancers, particularly in areas where the implementation of such practices is not ideal. While some case–control studies have shown that regular tooth brushing was associated with a lower risk of esophageal and gastric cancers compared with infrequent brushing (e.g., less than daily; refs. 25, 26), the literature is generally sparse and additional studies are required to elucidate the effects of tooth brushing on UGI cancer risk.

In this study, we aimed to examine the relationship between oral health and UGI cancers using data from the Golestan Cohort Study, a large prospective study of more than 50,000 subjects in Iran. Multiple measures of oral health, including frequency of tooth brushing, tooth loss, and the sum of decayed, missing, or filled teeth (DMFT) were used to obtain a detailed assessment of oral health.

Materials and Methods

Study population

The design of the Golestan Cohort Study has been described in detail previously (27). Briefly, a prospective cohort of 50,045 individuals between ages 40 and 75 years old were recruited from January 2004 to June 2008 in the Golestan Province of northeastern Iran. Subjects were excluded from the study if they had previously been diagnosed with UGI cancers, were unwilling to participate, or were temporary residents. Written informed consent was obtained from all participants at enrollment. This study was approved by the Institutional Review Boards of the Digestive Disease Research Institute of Tehran University of Medical Sciences (Tehran, Iran), the International Agency for Research on Cancer, and the U.S. NCI.

At the time of enrollment, trained interviewers administered a general lifestyle questionnaire to collect participant information on baseline age, sex, socioeconomic status, ethnicity, residence, education, cigarette use, and opium use. A food frequency questionnaire was used to evaluate fruit and vegetable intakes at baseline. Socioeconomic status was estimated using a composite wealth score based on ownership of vehicles, property, and household appliances, as described previously (28). In addition, trained staff measured the temperature at which participants consumed tea, because hot tea consumption is likely to be an important risk factor for ESCC in this population (29). All interviews were conducted in-person and there were no surrogate interviewees.

The reliability of questionnaire information from the Golestan Cohort Study has been reported previously (30). On the basis of a repeated interview of 130 subjects performed 2 months apart, the kappa statistic of self-reported cigarette smoking status and opium use status were 0.97 and 0.96, respectively. The high agreement between urinary cotinine positivity and self-reported smoking status was demonstrated using urine samples collected from 96 randomly selected participants (Pearson correlation coefficient = 0.73). In addition, the reliability of tea temperature measurements was also shown to be high, with a kappa statistic of 0.71. The validity of self-reported opium use has also been reported previously (31). The validity of self-reported opium use was assessed by comparing the questionnaire responses with the presence of codeine and morphine in urine samples collected from 150 participants. This analysis showed that the validity of self-reported opium use was high, with a concordance rate of 91% between self-report and urine measurements.

Oral health assessment

During the baseline interview, participants were asked about the frequency of tooth brushing (categorized as never, non-daily, and daily) and whether they owned partial or full dentures. The number of lost teeth and the sum total of DMFT were counted by trained medical personnel at baseline. When the numbers of teeth lost or DMFT exceeded 32, they were replaced with 32 to represent the total number of adult teeth. On the basis of the pilot study conducted for the Golestan Cohort Study, the reliability of tooth counts was high, with 88.3% agreement and a kappa statistic of 0.86 for repeated examinations of 130 subjects occurring 2 months apart (30). The reliability over time of self-reported brushing frequency was also assessed on the basis of a random sample of approximately 20% of the cohort (11,418 participants) who completed a repeat questionnaire (average of 5 years after baseline; ref. 32). Participants were once again asked how often they brush their teeth, and a comparison of the self-reported brushing frequency at baseline and from the repeated assessment showed excellent agreement with 77.9% concordance ($P < 0.001$).

Case ascertainment

All study participants were followed by annual telephone surveys or home visits to collect information about deaths and incident cancers. Provincial cancer and death registry databases were also reviewed monthly to identify cancer cases and deaths. When a cancer case or death was reported, trained staff visited the participant's home and the medical center where any diagnostic or therapeutic procedures were performed. All available clinical or pathology reports and hospital records were collected. In the case of death, a validated verbal autopsy was also performed, where a family member or a primary caregiver of the deceased participant were interviewed by a trained general practitioner to obtain information about the cause of death (33). Cancer cases and deaths

were independently verified by two physicians based on all available documents, and the date of outcome occurrence and the outcome code [according to the International Classification of Diseases, Tenth Revision (ICD-10)] were determined by each physician. When there was a disagreement in the disease codes between the two physicians, a third physician independently reviewed the documents and made the final diagnosis. In this analysis, gastric cancer cases were limited to adenocarcinomas (cardia and noncardia), and seven subjects diagnosed with nonepithelial malignancies (e.g., lymphoma, atypical carcinoid, or neuroendocrine carcinoma) in the esophagus or stomach were excluded.

Statistical analysis

Nine subjects missing oral health variables and 50 subjects with other missing covariates were excluded, leaving a total of 49,979 participants in the analysis. Because tooth loss and DMFT tend to be strongly correlated with age and sex, we used a loess model to calculate the age- and sex-specific predicted number of teeth lost and DMFT score (16, 34, 35). To do this, a loess model was fit to estimate predicted (defined as the median) number of lost teeth or DMFT score at each integer year of age using data stratified by sex. The difference between the actual and predicted number of lost teeth/DMFT score was calculated for each participant. Those with a difference less than or equal to 0 were categorized into one group, while the remaining subjects with excessive tooth loss or DMFT were categorized into quartiles. The smoothing parameter of the loess model was selected on the basis of the bias-corrected Akaike information criterion.

Cox proportional hazards regression models were used to estimate HRs and corresponding 95% confidence intervals (CI) for the association between oral health variables (i.e., tooth loss, DMFT, and frequency of tooth brushing categories) and the risk of esophageal and gastric cancers, with models looking at the two cancers separately and combined as UGI cancers. For the combined analysis of both UGI cancers, esophageal and gastric cancer cases were pooled and included in the same Cox model together. We also tested the association between oral health variables and UGI cancers by performing a meta-analysis with random effects models. Because the pooled analysis and the meta-analysis produced very similar results, we show only the estimates of HRs and 95% CIs from the pooled analysis as the results (meta-analysis results provided in Supplementary Table S1). The entry time was defined as the participants' age at enrollment. Follow-up was ended at the age of the first occurrence of one of the following events: diagnosis of esophageal or gastric cancer, diagnosis of other cancers, death from any cause, or last follow-up through December 31, 2019. A total of 505 participants (1.01%) were lost to follow-up during this period. The proportional hazards assumption was verified using the Schoenfeld residuals test. When the proportional hazards assumption was not fulfilled, we tested for time-varying covariates after identifying appropriate time cutoffs based on a plot of the scaled Schoenfeld residuals over time (36).

All Cox models were adjusted for the following variables: age, sex, socioeconomic score (in quartiles; ref. 28), ethnicity (Turkmen or non-Turkmen), residence (urban or rural), education (no formal education, $\leq$8 years, or >8 years), cigarette use (never, former, or current), and opium use (never or ever). For esophageal cancer, the Cox model was further adjusted for the temperature of hot tea consumption, which was categorized as <60°C, 60°C–64°C, and >65°C. Cox models testing the effect of tooth loss were also adjusted for denture use (yes or no). Additional adjustment for pack-years of cigarette use, the source of drinking water, and vegetable and fruit intake had no substantial effect (<10% change in HRs) on the association between the oral health variables and esophageal, gastric, or combined UGI cancers, so these variables were not included in the final models. Oral health variables were tested for a linear trend by assigning ordinal numbers to each category and a global trend using the Wald test. All statistical analyses were performed in the R statistical programing environment (version 3.6.1).

Data availability statement

The data that support the findings in this study are available from the corresponding author upon request.

Results

Baseline characteristics of the cohort overall and by frequency of tooth brushing is presented in **Table 1**. Most subjects were of Turkmen ethnicity (74.5%), had no formal education (70.2%), lived in rural areas (80.0%), and had never smoked cigarettes (82.7%) or used opium (83.0%; ref. 37). More than half of the participants reported never brushing their teeth (55.7%), and the overall mean number of lost teeth and DMFT were 18.3 and 23.4, respectively. During the median follow-up duration of 13 years, there were 396 incident cases of esophageal cancer (crude incidence rate of 64.7 per 100,000 person-years), among which 319 had ESCC, 14 had esophageal adenocarcinoma, and the remaining 63 did not have histologic identification. There were 398 incident cases of gastric cancer (crude incidence rate of 65.0 per 100,000 person-years), of which 182 were cardia and 216 were noncardia. Among the gastric cancer cases, 365 were adenocarcinomas and 33 did not have histologic identification. Combined, there were a total of 794 incident cases of UGI cancers.

Overall, daily tooth brushing was associated with a reduced risk of developing UGI cancers with a linear trend (**Fig. 1**); the HR for the association between daily brushing (vs. never brushing) and UGI cancer risk was 0.697 (95% CI: 0.558–0.871). Daily tooth brushing (vs. never) was associated with a significantly reduced risk for esophageal cancer (HR = 0.670; 95% CI: 0.486–0.924). For gastric cancer, daily tooth brushing (vs. never) was marginally associated with a lower risk (HR = 0.741; 95% CI: 0.544–1.01). However, the association between tooth brushing and gastric cancer demonstrated nonproportionality (P = 0.0377). The plot of the Schoenfeld residuals showed a

Table 1. Baseline characteristics of participants in the Golestan Cohort Study, overall and by frequency of tooth brushing.

			Frequency of brushing		
		Overall[a]	Never[b]	Nondaily[b]	Daily[b]
N (%)		49,979	27,836 (55.7)	8,339 (16.7)	13,804 (27.6)
Age, years, mean (SD)		51.6 (8.91)	53.6 (9.22)	49.0 (7.70)	48.9 (7.77)
Sex, *n* (%)	Female	28,779 (57.6)	15,115 (52.5)	4,527 (15.7)	9,137 (31.7)
	Male	21,200 (42.4)	12,721 (60.0)	3,812 (18.0)	4,667 (22.0)
SES, quartile, *n* (%)	Q1 (low SES)	13,912 (27.8)	10,208 (73.4)	1,690 (12.1)	2,014 (14.5)
	Q2	11,130 (22.3)	6,903 (62.0)	1,887 (17.0)	2,340 (21.0)
	Q3	12,576 (25.2)	6,517 (51.8)	2,379 (18.9)	3,680 (29.3)
	Q4 (high SES)	12,361 (24.7)	4,208 (34.0)	2,383 (19.3)	5,770 (46.7)
Ethnicity, *n* (%)	Turkman	37,210 (74.5)	21,211 (57.0)	6,389 (17.2)	9,610 (25.8)
	Non-Turkman	12,769 (25.5)	6,625 (51.9)	1,950 (15.3)	4,194 (32.8)
Residence, *n* (%)	Urban	10,010 (20.0)	3,936 (39.3)	1,503 (15.0)	4,571 (45.7)
	Rural	39,969 (80.0)	23,900 (59.8)	6,836 (17.1)	9,233 (23.1)
Education, *n* (%)	None	35,077 (70.2)	22,365 (63.8)	5,060 (14.4)	7,652 (21.8)
	Any	14,902 (29.8)	5,471 (36.7)	3,279 (22.0)	6,152 (41.3)
Cigarette use status, *n* (%)	Never	41,329 (82.7)	22,247 (53.8)	6,874 (16.6)	12,208 (29.5)
	Former	3,209 (6.42)	2,122 (66.1)	493 (15.4)	594 (18.5)
	Current	5,441 (10.9)	3,467 (63.7)	972 (17.9)	1,002 (18.4)
Opium use, *n* (%)	Never	41,504 (83.0)	21,653 (52.2)	7,112 (17.1)	12,739 (30.7)
	Ever	8,475 (17.0)	6,183 (73.0)	1,227 (14.5)	1,065 (12.6)
Hot tea temperature, *n* (%)	<60C	19,415 (38.8)	10,130 (52.2)	3,167 (16.3)	6,118 (31.5)
	60–64C	19,183 (38.4)	10,785 (56.2)	3,261 (17.0)	5,137 (26.8)
	>65C	10,816 (21.6)	6574 (60.8)	1,830 (16.9)	2,412 (22.3)
	No tea	565 (1.13)	347 (61.4)	81 (14.3)	137 (24.2)
Fruits and vegetables intake, *grams/day*, mean (SD)[c]		336 (179)	320 (173)	335 (170)	368 (192)
Denture use, *n* (%)	No	34,452 (68.9)	20,777 (60.3)	5,712 (16.6)	7,963 (23.1)
	Yes	15,527 (31.1)	7,059 (45.5)	2,627 (16.9)	5,841 (37.6)
Number of teeth missing, mean (SD)		18.3 (9.55)	20.3 (9.04)	15.2 (8.96)	16.0 (9.87)
Tooth loss group, *n* (%)	Expected or less	26,328 (52.7)	12,384 (47.0)	5,408 (20.5)	8,536 (32.4)
	Q1	6,753 (13.5)	4,391 (65.0)	984 (14.6)	1,378 (20.4)
	Q2	5,384 (10.8)	3,786 (70.3)	613 (11.4)	985 (18.3)
	Q3	6,470 (12.9)	4,221 (65.2)	734 (11.3)	1,515 (23.4)
	Q4	5,044 (10.1)	3054 (60.5)	600 (11.9)	1,390 (27.6)
Number of decayed teeth, mean (SD)		4.95 (6.05)	5.02 (6.06)	5.30 (5.85)	4.60 (6.12)
Number of filled teeth, mean (SD)		0.220 (1.15)	0.0569 (0.715)	0.196 (1.02)	0.564 (1.72)
DMFT score, mean (SD)		23.4 (8.73)	25.4 (7.92)	20.7 (8.80)	21.1 (9.24)
DMFT group, *n* (%)	Expected or less	22,265 (44.5)	10,062 (45.2)	4,756 (21.4)	7,447 (33.4)
	Q1	10,066 (20.1)	6,736 (66.9)	1,291 (12.8)	2,039 (20.3)
	Q2	5,825 (11.7)	3,831 (65.8)	716 (12.3)	1,278 (21.9)
	Q3	5,081 (10.2)	3,191 (62.8)	600 (11.8)	1,290 (25.4)
	Q4	6,742 (13.5)	4,016 (59.6)	976 (14.5)	1,750 (26.0)

[a]Percentages are columnwise.
[b]Percentages are rowwise.
[c]Numbers missing in 870 subjects.

nonconstant effect of tooth brushing over time, with changes in the effect occurring at approximately 4 and 7 years of follow-up (Supplementary Fig. S1). Therefore, time-varying covariates were created to look at the effects of tooth brushing during the following time intervals: ≤4 years (early), >4 and ≤7 years (mid), >7 years (late). This showed that the protective effect of tooth brushing on gastric cancer risk was most prominent during the early phase with a HR of 0.458 (95% CI: 0.229–0.918) for daily brushing (vs. never), but associations weakened and became null thereafter (Supplementary Table S2).

The highest quartile of tooth loss was associated with a significantly increased risk for UGI cancers, compared with subjects with the expected or fewer than expected number of teeth lost based on sex and age (**Fig. 2**). For esophageal cancer, subjects in the highest quartile of teeth lost was associated with a HR of 1.64 (95% CI: 1.08–2.47) compared with subjects with the expected or fewer number of teeth lost. Similarly, the HR for the highest quartile of tooth loss was 1.58 for gastric cancer (95% CI: 1.05–2.38). There was a significant linear trend across quartiles of tooth loss for both UGI cancers, where the risk increased with an increase in teeth lost.

The relationship between DMFT and UGI cancer risk was less clear (**Fig. 3**). The third quartile of DMFT was significantly associated with a 1.46-times (95% CI: 1.05–2.03) higher risk of esophageal cancer compared with subjects with DMFT scores as expected or less, but there was no association with the highest

	Brushing	Cases (*n*)	HR	95% CI	*P*
Esophagus	Never	302	Reference		
	Nondaily	46	0.839	(0.610–1.15)	0.281
	Daily	48	0.670	(0.486–0.924)	0.0146
	Linear trend		0.822	(0.705–0.957)	0.0117
	Global trend				0.0419
Stomach	Never	301	Reference		
	Nondaily	43	0.778	(0.560–1.08)	0.135
	Daily	54	0.741	(0.544–1.01)	0.0570
	Linear trend		0.850	(0.731–0.987)	0.0335
	Global trend				0.0858
UGI	Never	603	Reference		
	Nondaily	89	0.805	(0.640–1.01)	0.0640
	Daily	102	0.697	(0.558–0.871)	0.00147
	Linear trend		0.831	(0.747–0.925)	0.000684
	Global trend				0.00290

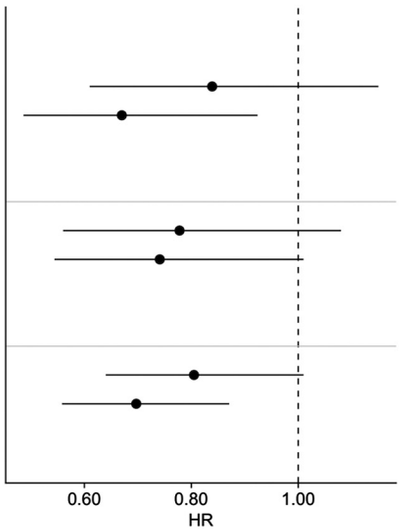

Figure 1.

Associations between frequency of tooth brushing and cancers of the esophagus, the stomach, and the two sites combined [i.e., UGI series]. The dotted line represents HR = 1.00. All models were adjusted for age, sex, socioeconomic score, ethnicity, residence, education, cigarette use, and opium use. Associations with esophageal cancer were also adjusted for tea temperature.

quartile. For gastric cancer, there were no associations for all quartiles of DMFT.

Sensitivity analyses excluding the first 2 years of follow-up (Supplementary Tables S3–S5) and restricting the analysis to confirmed ESCC cases did not change the results substantially (Supplementary Table S6). When the analysis was performed separately for gastric cardia and noncardia cancers, daily tooth brushing showed a significantly reduced risk for gastric cardia cancer compared with never brushing (HR = 0.621; 95% CI: 0.399–0.968), but not for noncardia gastric cancer (Supplementary Table S7). The highest quartile of tooth loss was not associated with gastric cardia cancer but showed a significantly increased risk for gastric noncardia cancer (HR = 1.98; 95% CI: 1.12–3.52; Supplementary Table S8). DMFT was not associated with gastric cardia or noncardia cancer (Supplementary Table S9). Moreover, while we explored

	Tooth loss	Cases (*n*)	HR	95% CI	*P*
Esophagus	Expected or less	177	Reference		
	Q1	58	1.04	(0.773–1.41)	0.785
	Q2	55	0.960	(0.693–1.33)	0.806
	Q3	68	1.27	(0.926–1.76)	0.137
	Q4	38	1.64	(1.08–2.47)	0.0189
	Linear trend		1.09	(1.00–1.19)	0.0431
	Global trend				0.127
Stomach	Expected or less	195	Reference		
	Q1	51	0.895	(0.656–1.22)	0.485
	Q2	52	1.07	(0.778–1.48)	0.663
	Q3	63	1.25	(0.905–1.72)	0.177
	Q4	37	1.58	(1.05–2.38)	0.0281
	Linear trend		1.09	(1.00–1.19)	0.0404
	Global trend				0.145
UGI	Expected or less	372	Reference		
	Q1	109	0.971	(0.783–1.20)	0.787
	Q2	107	1.02	(0.813–1.28)	0.853
	Q3	131	1.27	(1.01–1.59)	0.0413
	Q4	75	1.61	(1.21–2.16)	0.00123
	Linear trend		1.10	(1.03–1.16)	0.00347
	Global trend				0.0106

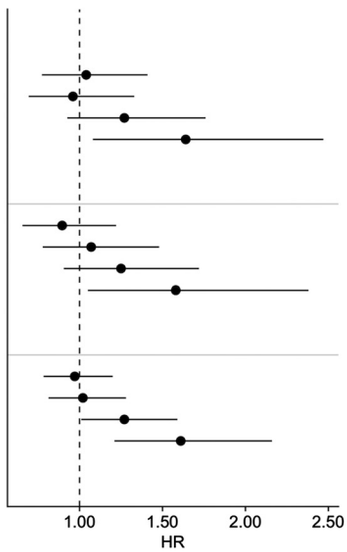

Figure 2.

Associations between tooth loss and cancers of the esophagus, the stomach, and the two sites combined [i.e., UGI series]. The dotted line represents HR = 1.00. All models were adjusted for age, sex, socioeconomic score, ethnicity, residence, education, cigarette use, opium use, and denture use. Associations with esophageal cancer were also adjusted for tea temperature.

	DMFT	Cases (*n*)	HR	95% CI	*P*
Esophagus	Expected or less	151	Reference		
	Q1	113	0.901	(0.692–1.17)	0.438
	Q2	55	1.01	(0.738–1.37)	0.966
	Q3	49	1.46	(1.05–2.03)	0.0228
	Q4	28	0.960	(0.626–1.47)	0.853
	Linear trend		1.04	(0.958–1.13)	0.349
	Global trend				0.111
Stomach	Expected or less	162	Reference		
	Q1	102	0.857	(0.656–1.12)	0.257
	Q2	55	0.942	(0.691–1.28)	0.704
	Q3	37	1.04	(0.724–1.49)	0.836
	Q4	42	1.25	(0.870–1.80)	0.225
	Linear trend		1.03	(0.954–1.12)	0.432
	Global trend				0.518
UGI	Expected or less	313	Reference		
	Q1	215	0.879	(0.728–1.06)	0.177
	Q2	110	0.972	(0.781–1.21)	0.801
	Q3	86	1.24	(0.977–1.58)	0.0771
	Q4	70	1.12	(0.848–1.48)	0.426
	Linear trend		1.04	(0.979–1.10)	0.224
	Global trend				0.153

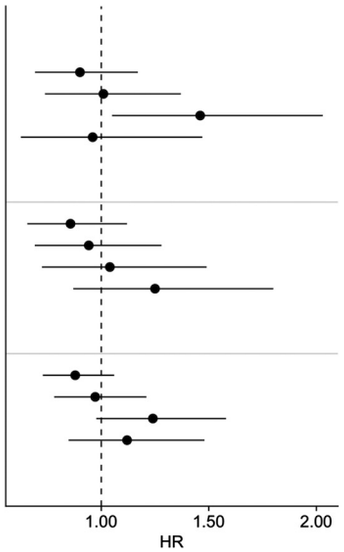

Figure 3.

Associations between DMFT and cancers of the esophagus, the stomach, and the two sites combined [i.e., UGI series]. The dotted line represents HR = 1.00. All models were adjusted for age, sex, socioeconomic score, ethnicity, residence, education, cigarette use, and opium use. Associations with esophageal cancer were also adjusted for tea temperature.

differences in associations by sex, we found no evidence of an interaction between sex and any of the oral health measures ($P > 0.100$).

Discussion

In this large prospective study from a high-incidence region for UGI cancers, daily tooth brushing was associated with an approximately 30% lower risk for UGI cancers compared with subjects who never brushed their teeth. In addition, the highest quartile of tooth loss was associated with an approximately 60% higher risk for both esophageal and gastric cancers compared with subjects with the expected number of teeth lost or fewer. These associations persisted after adjusting for other previously identified risk factors in this population, including cigarette and opium smoking and hot tea consumption (29, 35).

Few studies, mostly consisting of case–control studies, have examined the effect of tooth brushing on UGI cancer risk (25). Our results concur with previous case–control studies from Golestan that showed protective effects of tooth brushing for UGI cancers (26, 38, 39). A recent cohort study in Korea has also shown that brushing teeth at least three times a day was associated with a 14% lower risk for gastric cancer compared with brushing 0–1 times a day, but they did not find an association for esophageal cancer (23). In Korea, 85.3% of the study population reported brushing at least twice a day (23), whereas more than half of the participants in Golestan reported never brushing their teeth. It is possible that stronger effects were observed for both UGI cancers in our study because the frequency of tooth brushing was more varied in Golestan compared with Korea. While more evidence is required, these results suggest that tooth brushing may help decrease the risks for UGI cancers, particularly in populations where regular oral hygiene practices are uncommon.

Lack of regular oral hygiene can result in periodontal disease, caries, and tooth loss. Similar to our findings, in a large prospective study conducted in Linxian, China, another high-incidence region for UGI cancers, tooth loss was associated with a HR of 1.3 for esophageal and gastric cardia cancers and 1.8 for gastric noncardia cancer (15). Our results also showed a stronger association between tooth loss and gastric noncardia compared with gastric cardia cancer. Furthermore, it is unlikely that there was residual confounding due to smoking and alcohol intake for associations observed in this study, because smoking is not an important risk factor in this population and alcohol consumption is uncommon in Golestan (5, 40, 41).

However, there are also a few previous studies from Western countries, which have shown no associations between tooth loss and UGI cancers. In a prospective study of male health professionals in the United States, the association between tooth loss and esophageal and gastric cancers was null, after adjusting for major confounders such as smoking (22). Similarly, no association was found between UGI cancers and self-reported measures of poor oral health (e.g., painful or bleeding gums, loose teeth) in a cohort study from the United Kingdom (24). The reasons for the discrepancies in the results remain unclear, but heterogeneity across populations in the underlying risk factors for UGI cancers, based on local habits and the prevalence of these risk factors within the population, may have played a role (2). Therefore, the importance of poor oral health as a risk factor for UGI cancers may differ between Western countries and high-risk areas such as Golestan and Linxian. In addition, poor dentition is more common in

Golestan and Linxian (42) compared with the United States and United Kingdom, and the greater degree of variation in tooth loss may have made risk associations easier to detect in these high-risk areas. It is also possible that the primary reasons for tooth loss vary, and some populations may experience tooth loss due to other reasons besides periodontal status and poor oral hygiene (e.g., trauma, lack of access to dental services; ref. 43). Moreover, the suboptimal levels of drinking water fluoride concentrations in Golestan province may contribute to poor oral health (44, 45), but additional studies are required to explore this possibility.

Although the mechanism for the association between oral health and UGI cancers is unknown, it is hypothesized that the oral microbiome may be involved because tooth loss often results from periodontal disease, which is caused by specific bacterial pathogens in the oral cavity (46). Oral bacteria are constantly swallowed with saliva and delivered to the digestive tract, including the esophagus and stomach. In fact, periodontal pathogens, particularly anaerobic species such as *Porphyromonas gingivalis* and *Treponema denticola*, stimulate chronic inflammation, which can contribute to carcinogenesis (47), and these oral pathogens have been detected in esophageal and gastric cancer tissues (48, 49). Oral bacteria can also produce carcinogenic metabolites, such as *Streptococcus mutans* that reduces nitrate to nitrite, precursors to nitrosamines that have been linked to esophageal cancer (15, 50, 51). The protective effects of tooth brushing may be due to the removal of dental plaque, a biofilm consisting of microorganisms including bacteria, which can aid in the prevention of periodontal disease (52).

This study has several important strengths and limitations. The strengths include the large sample size, the prospective design, and a low proportion of subjects lost to follow-up. Instead of using self-reported tooth loss and DMFT, oral health was assessed by trained medical personnel to obtain reliable measures of these variables. We also used a loess model to minimize the confounding effect of age on tooth loss and DMFT measures. However, measurement error is still possible, particularly for DMFT, because decay was assessed without a full-mouth dental exam by a dentist. Although our medical personnel received training before the study, diagnosis of decay may require more technical experience and specific tools such as imaging. In addition, all oral health measures were only assessed only at baseline and did not account for changes during follow-up. We also did not account for periodontal status or other potential causes of tooth loss that may be an important unmeasured confounder. *H. pylori* seropositivity was not included in our analysis so we cannot rule out the possibility of confounding by *H. pylori* infection, although this is unlikely because the prevalence of this infection is very high and nearly ubiquitous in Golestan (8).

Conclusion

In conclusion, we found that daily tooth brushing showed protective effects for both esophageal and gastric cancers, and higher numbers of teeth lost significantly increased the risk of both UGI cancers in a large prospective cohort from a high-risk region. Additional research is required to understand the underlying mechanisms, which may involve alterations in the oral microbiota.

Authors' Disclosures

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Authors' Contributions

Y. Yano: Conceptualization, data curation, software, formal analysis, methodology, writing–original draft. **C.C. Abnet:** Conceptualization, formal analysis, methodology, writing–original draft, project administration. **H. Poustchi:** Resources, investigation, writing–review and editing. **G. Roshandel:** Data curation, investigation, writing–review and editing. **A. Pourshams:** Investigation, writing–review and editing. **F. Islami:** Investigation, project administration, writing–review and editing. **M. Khoshnia:** Investigation, writing–review and editing. **T. Amiriani:** Investigation, writing–review and editing. **A. Norouzi:** Investigation, writing–review and editing. **F. Kamangar:** Methodology, project administration, writing–review and editing. **P. Boffetta:** Project administration, writing–review and editing. **P. Brennan:** Project administration, writing–review and editing. **S.M. Dawsey:** Project administration, writing–review and editing. **E. Vogtmann:** Data curation, writing–review and editing. **R. Malekzadeh:** Resources, supervision, investigation, project administration, writing–review and editing. **A. Etemadi:** Conceptualization, data curation, software, formal analysis, supervision, investigation, methodology, writing–original draft, project administration.

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