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Food Preparation Methods, Drinking Water Source and Esophageal Squamous Cell Carcinoma in the High Risk Area of Golestan, Northeast Iran

Asieh Golozar^{1,2,3}, Arash Etemadi^{1,2}, Farin Kamangar^{1,4}, Akbar Fazeltabar Malekshah¹, Farhad Islami^{5,1}, Dariush Nasrollahzadeh^{6,1}, Behnoosh Abedi-Ardekani^{1,7}, Masoud Khosnia⁸, Akram Pourshams¹, Shahriar Semnani⁸, Haji Amin Marjani^{8,1}, Ramin Shakeri¹, Masoud Sotoudeh¹, Paul Brennan⁷, Philip Taylor², Paolo Boffetta^{5,9}, Christian Abnet², Sanford Dawsey², and Reza Malekzadeh¹

¹Digestive Disease Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

²Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA

³Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

⁴School of Community Health and Policy, Morgan State University, Baltimore, MD, USA

⁵The Tisch Cancer Institute and Institute for Translational Epidemiology, Mount Sinai School of Medicine, New York, USA

⁶Medical epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

⁷International Agency for Research on Cancer, Lyon, France

⁸Golestan Research Center of Gastroenterology and Hepatology, Gorgan University of Medical Sciences, Gorgan, Iran

⁹International Prevention Research Institute, Lyon, France

Abstract

Food cooking practices and water sources have been associated with increased risk of cancer, mainly through exposure to carcinogens such as heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs) and nitrates. Using data from the Golestan case-control study, conducted between 2003 and 2007 in a high-risk region for esophageal squamous cell carcinoma (ESCC), we sought to investigate the association between food preparation and drinking water sources and ESCC. Information on food preparation methods, sources of drinking water and dietary habits were gathered from 300 cases and 571 controls individually matched for age, sex and neighborhood using a structured questionnaire and a semi-quantitative food frequency questionnaire, respectively. Multivariate conditional logistic regression was used to estimate odds

Corresponding Author: Dr Asieh Golozar, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 605 N. Wolfe St, E6036, Baltimore, MD, USA. Phone: 443-287-8777, agolozar@jhsph.edu.

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ratios (OR) adjusted for potential confounders and other known risk factors including socioeconomic status and smoking. More than 95% of the participants reported using meat, mostly red meat. Red meat consumption above 75th percentile increased the odds of ESCC by 2.1 fold (95% confidence interval (CI): 1.2, 3.6). On the other hand, fish intake was associated with a significant 70% decrease in ESCC odds (0.4, 0.8). Among meat users, ORs (95% CI) for frying meat (red or white) and fish were 2.9 (1.6, 5.7) and 2.3 (1.3, 4.1), respectively. Drinking unpiped water increased the odds of ESCC by 4.3 times (2.2, 8.1). The OR for each 10-year increase in the duration of drinking unpiped water 1.5 (1.2, 1.8). Our results suggest independent roles for red meat intake, drinking water source, and food preparation method in ESCC.

Keywords

Dietary Habits; Food preparation; Cooking Methods; Esophageal Squamous Cell Carcinoma; Water Sources; Environmental Exposure; Environmental Carcinogens

Introduction

The so-called “Asian Esophageal Cancer Belt” which stretches from northcentral China to northeastern Iran, houses some of the highest rates of esophageal squamous cell carcinoma (ESCC), the most common type of esophageal cancer (1). No single dominant risk factor has been identified in these high-risk regions, and alcohol and tobacco, the major ESCC risk factors in low-risk areas appear to have notably less effect in these populations (2–4). The similarity of incidence rates of ESCC in both sexes implies a role for exposures affecting both sexes equally in the etiology of ESCC (1, 5).

A role for diet in the etiology of ESCC in high risk areas has been implicated since the early 1970s (6–9). While the association between low intake of fresh fruits and vegetables and ESCC has been consistently supported by epidemiologic studies, evidence suggesting a role for animal based foods (red meat, poultry and fish) in ESCC etiology is limited (10–12). Food and water are also potential sources of some of the carcinogens that are possibly involved in the etiology of ESCC, including heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs) and nitrates (8, 9, 13). Food cooking practices are among the possible mechanisms through which dietary habits could increase the risk of ESCC (13). Certain cooking methods have been associated with the formation of HCAs and PAHs, both shown to be involved in the carcinogenic process of different cancers including lung, colon and skin (13–17). Ingestion of HCAs and PAHs has shown to induce gastrointestinal (GI) tract tumors in animal models [*Cancer Sci.* 2004 Apr;95(4):290–9. *Carcinogenesis*. 2003 Mar;24(3):613–9.] (18, 19). The increased risk of ESCC associated with red meat intake may be partially explained by the PAHs and HCAs red meat is exposed to during cooking (14, 20).

The source of drinking water has been consistently implicated in ESCC incidence and mortality in high-risk areas of Iran and China (3, 5, 8). This association has been attributed to contamination of water with several biological and chemical pollutants including nitrates. Several ecological and epidemiological studies have suggested a link between high nitrate content of water and GI cancers, including ESCC (21–23).

Accurate estimation of the intake of such carcinogens is challenging in epidemiologic studies. Assessing contact to sources of such exposures including meat intake, cooking methods and source of drinking water might serve as a good proxy of these exposures. In this study, we used data from the Golestan Case-Control study, conducted in a high-risk area in northeastern Iran, to assess the association between food preparation and drinking water sources and ESCC.

Methods and Materials

Study Population

Details of the Golestan Case-Control study have been reported previously (2). Between December 2003 and June 2007, individuals in eastern Golestan Province with signs and symptoms suggestive of Upper GI cancer were referred to Atrak Clinic, the only GI referral clinic in that region to undergo esophagogastroscope. Biopsy samples obtained during the procedure were reviewed by expert pathologists at the Digestive Disease Research Center (DDRC) of Tehran University of Medical Sciences. Histologically proven ESCC cases who were more than 18 years old, resided in the eastern Golestan at the time of the study, had no previous history of any other cancer and consented to participate in the study were included. For each case, two neighborhood controls individually matched for age (± 2 years) and sex were enrolled. Family health census was used to develop a roster of eligible controls for each case both in rural and urban areas. In urban area, controls with the closest proximity to the cases were selected at random from the roster, and in rural areas, controls were selected randomly from the same village. If the first person could not participate, the second random person on the list was approached. Approximately 80% of controls were the first randomly selected persons. Absence of the eligible control was the reason for not participating in the study in nearly all the instances. For ~10% of cases, only one control could be recruited.

Data Collection

Participants were interviewed by trained physicians and nutritionists able to communicate in either Farsi (the national language), or Turkmen (the local language).

Lifestyle information—Demographics and baseline information were collected using a structured lifestyle questionnaire. Education, defined as the highest level of education attained, was divided into three groups: no education, primary school, and middle school or higher. Appliances ownership variables were used to create a wealth score using multiple correspondence analysis and participants were categorized into of wealth score tertiles: low, middle and high. Education and wealth score categories were used as indicators of socioeconomic status (SES) (24).

Weight (kg), and height (cm) were measured to the nearest 0.5 kg and 0.1 cm, respectively. Body mass index (BMI) was calculated by dividing measured weight (kg) by the square of the measured height (m), and categorized using the World Health Organization (WHO) cutoffs: underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$), normal ($18.5 \leq \text{BMI} < 25 \text{ kg/m}^2$), overweight ($25 \leq \text{BMI} < 30 \text{ kg/m}^2$), and obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) (32).

Lifelong history of tobacco and opium use was collected separately. Information on the starting and quitting age and the amount used each time were recorded for those using either product. Individuals were considered tobacco users if they had smoked cigarettes or had used nass, hookah or a pipe at least once a week for more than 6 months.

Individuals were categorized into these groups: never smokers, former cigarette smokers, current cigarette smokers, and those who smoked other forms of tobacco (nass, hookah, or a pipe). Likewise, opium users were defined as those who consumed opium at least once a week for 6 months or more.

All participants were asked to describe the temperature of their tea when they drink it. Tea drinking temperature was categorized into warm, lukewarm, hot, or very hot [ref Islami].

Caries experience (the sum of the number of decayed, missing, or filled teeth (DMFT)) was used as an indicator of oral hygiene. The DMFT score was then categorized into 4 levels: <16, 16 and <23, 23 and <27, 27 and <32, and 32. Frequency of experiencing eating discomfort was categorized into three groups: most of the time, often and rarely.

Information on the source of drinking water was gathered from all participants. Drinking water source was categorized into piped and unpiped water. Unpiped sources included wells, surface water, cisterns, etc. Duration of using piped water and the prior source of drinking water was also asked from individuals reporting piped water use.

Participants were asked about different meat (red and white), fish and vegetable cooking practices (deep or shallow frying, barbecuing, steaming and boiling).

Dietary information—Dietary habits were collected from all participants at enrollment, using a validated food frequency questionnaire (FFQ) specifically developed for this population (25). The FFQ contained 115 single food items. For each food item consumed, participants were asked to indicate the typical portion size, consumption frequency, and the number of servings. Daily intake of each item was calculated by multiplying the consumption frequency by the typical portion size and the number of servings. Total daily consumption of red meat, poultry, fish and vegetable was calculated by summing up the item-specific score of each food group. Red meat intake included beef, lamb, game and camel. Poultry intake included chicken and gizzard and fish intake included sturgeon, dace and freshwater fish. Participants who reported using red meat were categorized into quartiles of daily red meat intake in controls (<107, 108–175, 176–268 and >269 grams/day). Poultry and fish intake were not commonly reported in this population. Thus, we categorized participants into consumers and non-consumers of poultry or fish. Processed meat/fish included any meat or fish that have undergone preservation methods other than freezing such as salting (with or without nitrites), smoking, air drying or heating. Since processed meat/fish intake was very infrequent in Golestan, we did not include it in our final analysis.

The Golestan Case-Control Study was approved by the Institutional Review Boards of the DDRC of Tehran University of Medical Sciences, the US National Cancer Institute, and the World Health Organization's International Agency for Research on Cancer, and all participants gave written informed consent before enrollment.

Statistical Analysis

Normally and non-normally distributed continuous variables are presented as means ($\pm$ standard deviation (SD)) and median (interquartile range (IQR)). The Student's t-test and the non-parametric Wilcoxon Rank sum test were used to test association between ESCC and these variables, respectively. Categorical variables are presented as numbers (percentages). The relationship between categorical variables and ESCC was tested using the chi-square test.

We used conditional logistic regression to calculate odds ratio (OR)s and their corresponding 95% confidence intervals (CI) for food- and water-related exposures. The association between frying fish and meat (red or white) were only studied among those consuming fish or meat, respectively.

We found no evidence of clear multicollinearity as the variance inflation factors were all below 1.5. Separate multivariate models were then fit to assess the association between food- and water-related exposures and ESCC. The food-related exposure models were restricted to participants who consumed some red meat, poultry or fish. All models were further adjusted for potential confounding of ethnicity, education, wealth score, opium use, tobacco use, family history of ESCC, BMI categories, tea drinking temperature, vegetable intake, DMFT score and frequency of eating discomfort. All tests of hypothesis were conducted at confidence level 0.95 under two-sided alternatives.

Results

Three hundred confirmed ESCC cases and 571 controls individually matched for age and sex were included in the analyses. The mean (SD) age of the study participants was 64.3 (10.7), ranging from 30 to 88. The male to female ratio was 0.97 and men were on average 2.0 ($\pm$ 0.7) years older than women. Nearly one-quarter of the participants were of urban residence and 55.5% were of Turkmen ethnicity. Demographics and other characteristics of the ESCC cases and controls are presented in Table 1.

Vegetable consumption was reported by 95.6% of the study participants. The mean (SD) daily vegetable consumption was close in cases and controls (204.96 grams/day (133.58) vs. 193.28 grams/day (149.56); P-value=0.24).

Red meat was the most common form of meat consumed; 90.1% of all participants reported red meat consumption. The quantity of red meat consumed by cases was higher (*P-value* for the Wilcoxon ranksum test<0.001). The median (IQR) daily consumption of red meat was 15.2 grams/day (4.3, 35.4) in ESCC cases and 8.5 grams/day (2.8, 21.0) in controls, respectively (P-value<0.001). In the adjusted model, compared to red meat consumption below 25th percentile (quartile 1), red meat consumption above 75th percentile (quartile 4) was associated with a significant increase in the odds of ESCC (OR (95% CI): 2.8 (1.2, 6.6)). We also observed a significant stepwise increase in the odds of ESCC across quartiles of red meat intake (p-value for trend<0.05). Unlike red meat, poultry and fish intake adjusted for level of red meat intake were inversely associated with ESCC. There was a 33% non-

significant and 68% significant decrease in the odds of ESCC associated with consumption of poultry and fish, respectively (Table 2).

Frying in general was reported more commonly by cases than controls (92.6% vs. 87.0%; P -value<0.001). In those consuming some sort of meat or fish, the odds of ESCC increased by 3.3 (1.3, 8.5) times in those frying meat (red or white) and 2.6 (1.2, 5.6) times in those frying fish, respectively. The crude and adjusted estimated ORs (95% CI) for the meat and fish related exposures are presented in Table 2.

Drinking unpiped water was associated with an increased odds of ESCC; we observed a 4.3 time increase (2.2, 8.1) in the odds of ESCC associated with drinking unpiped water, and a significant 47% increase (22%, 78%) in the odds of ESCC associated with every 10-year increase in the duration of using unpiped water (Table 2).

Discussion

In this population-based case-control study, we observed a direct association between red meat consumption and ESCC. Adjusted for the level of red meat intake, fish and poultry intake, were inversely associated with ESCC. Frying meat and fish were both associated with increased likelihood of ESCC. Finally, ESCC was positively associated with drinking unpiped water and the duration of using unpiped water.

Red meat intake has been shown to be associated with increased risk of cancer in different organs including colon, lung, stomach and esophagus (17–19). Unlike colorectal cancer, the evidence supporting a role for red meat in upper GI cancer is limited (10). Results of population based prospective studies in low risk areas and case-controls studies in high-risk areas all consistently show a positive association between red meat intake and ESCC (19, 26). Like other reports, we observed an increase in the odds of ESCC associated with highest daily consumption of red meat.

The exact mechanism through which red meat is involved in carcinogenesis is not well understood yet. However, the heme iron, saturated fat content of the red meat and contamination with carcinogenic compounds including HCAs and PAHs, which are formed during high temperature cooking, have been suggested to play a role in the carcinogenic process (27, 28). The individual roles of the proposed mechanisms in ESCC carcinogenicity have not been thoroughly investigated (19). A positive association between heme iron intake and adenocarcinoma of the esophagus, but not ESCC, has been reported (27).

Poultry and fish consumption have been shown to be associated with lower risk of cancer. Results of the National institute of Health (NIH) AARP Diet and Health study indicated a more than 10% reduction in ESCC risk for every 10 grams increase in poultry and fish intake (29). A case-control study conducted in Uruguay, a high-risk area for ESCC, also reported a decrease in ESCC odds with increased consumption of poultry and fish (30). For individual evaluations of poultry and fish, however, the results are conflicting. While fish intake alone was not associated with ESCC risk in the AARP study (29), other studies have reported a strong protective effect for fish intake (26, 30). In our study, only fish intake was significantly associated with a reduction in ESCC odds. The anti-inflammatory properties of

omega-3 fatty acids and alpha-linolenic acids have been proposed as one of the plausible mechanisms for the observed protective effect of fish intake (31). Poultry and fish intake may also be a substitution for red meat intake and thus, the negative association might be explained by a concurrent lower intake of red meat, and lower heme iron and saturated fat content of the white meat and fish (32). In our study, adjusting for meat intake strengthened the negative association between fish intake and ESCC, so this substitution is unlikely to play a significant role in this protective association. Fish and poultry intake may also be a surrogate of a healthier eating pattern and lifestyle and a higher SES. Although we adjusted for two SES indicators previously shown to have a strong influence on ESCC risk, residual confounding from other SES-related factors and lifestyle patterns not captured in our study cannot be ruled out.

A positive association between meat cooked at high temperature and ESCC has been reported previously (26, 30, 33). In a previous study, we observed a higher level of deep frying in people from the high-risk area of Golestan (33). Consistent with the results of the case-control study in Uruguay, we observed associations between frying meat and fish and ESCC (26). HCA contamination of meat during high temperature frying has been suggested as possible mechanistic explanations for this association (13, 14, 20). Compared to other cooking methods, some of the highest levels of HCAs are formed during high temperature frying (13, 20). The oral ingestion of HCAs has been shown to induce upper GI cancers in animal studies (34). HCA (MeIQx and DiMeIQx) intake was also associated with an increased ESCC risk in a case-control study in Uruguay. (35)

PAHs are also believed to play a role in esophageal cancer causation even among non-smokers (36). There is a striking dose-response relationship between the PAH content of non-tumoral esophageal biopsies, and ESCC (37). Previous studies have shown very high levels of urinary 1-hydroxypyrene glucuronide (1-OHPG), a stable short-term PAH metabolite in high-risk areas of Iran and China (38, 39). The level of PAH-related DNA adducts is also very high in Golestan, even among female non-smokers (36). Hakami et al. have shown an increased amount of PAH intake from staple food in Golestan, which may contribute to this high exposure (40). Elevated levels of carcinogenic PAH in the kitchen air and meat following frying meat have been reported elsewhere (14). A positive association between red meat intake and urinary 1-OHPG has also been reported in high-risk areas of Golestan (41).

Drinking water is another potential route of exposure to different carcinogens such as nitrates, phosphates, oil and heavy metals (42). Several studies highlight a role for high levels of nitrate in drinking water in ESCC etiology, possibly through the formation of N-nitroso compounds (NOCs) (8). Compared to low-risk areas, drinking water contains more nitrate in the high-risk areas of Golestan (23), consistent with a role for nitrate-contaminated water in ESCC etiology. Old unpiped water sources, such as wells and cisterns, in this region contain higher amount of nitrates, even compared with newer piped water sources in the same neighborhood. In their ecological study, Keshavarzi et al. also showed that the content of nitrates, sulfates, and some minerals in drinking water correlate with esophageal cancer mortality rate (23). The association between unpiped water source and ESCC risk has also been shown in Linxian, China, another high-risk region for ESCC (3). Although access to

safe drinking water can be a surrogate of SES, the very strong and consistent association between drinking water source and ESCC even after controlling for the confounding effects of SES, suggests that the observed association cannot be solely due to SES. And in our study, since cases and controls were matched on neighborhood, this association is unlikely to reflect regional variations in ESCC incidence. The dose-response relationship between duration of drinking unpiped water and ESCC further supports this hypothesis.

Our study has some limitations, some of which are common to all case-control studies. Due to the retrospective design of this study, we were not able to establish temporal relationships between the assessed exposures and ESCC. However, some of the exposures, such as water source are unlikely to have been affected by recall bias or reverse causation. Also, meat intake or frying food is not commonly believed by the people to affect ESCC risk, so it is unlikely that recall affect the observed association. Moreover, ESCC patients are more likely to reduce rather than increase meat intake, because of difficulty swallowing, so any potential bias due to the reverse causation is expected to occur in the opposite direction of the observed results. Measurement error in dietary intake could have affected our findings, and is an integral part of any study relying on dietary assessment tools. We were also not able to separate the effect of frying poultry from red meat, because they were reported together.

Some of strengths of the study include histological validation of ESCC cases, use of a structured lifestyle questionnaire and a valid and reliable FFQ, the opportunity to assess the associations between cooking methods and water sources and ESCC, which are relatively understudied, and adjustment for multiple confounders, including several SES measures.

Our findings warrant further investigations and can be of great public health importance since they indicate the role of modifiable dietary and lifestyle habits that are widespread in this population as putative risk factors for ESCC. Preventive measures such as educating people about the dangers of high temperature frying, and the benefits of eating fish and reducing red meat intake, and societal efforts to increase access to safe drinking water are all realistic interventions which could have a significant public health impact.

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Table 1

Characteristics of cases and controls in the Golestan Case-Control Study of esophageal squamous cell carcinoma (ESCC) in Golestan, Iran

	Control, (N=571)	Case, (N=300)	P-value
Age, mean (SD)	64.27 (10.43)	64.48 (11.15)	--
Sex, N (%)			
Female	293 (51.31)	150 (50.00)	--
Male	278 (48.69)	150 (50.00)	
Ethnicity, N (%)			
Turkmen	312 (54.64)	171 (57.00)	0.018
Non-Turkmen	259 (45.36)	129 (43.00)	
Place of residence, N (%)			
Urban	150 (26.27)	82 (27.33)	0.700
Rural	421 (73.73)	218 (72.67)	
Education, N (%)			
None	474 (83.01)	267 (89.00)	0.040
Primary School	64 (11.21)	25 (8.33)	
Middle School or above	33 (5.78)	8 (2.67)	
Wealth Score, N (%)			
Low	198 (34.68)	93 (31.00)	0.400
Middle	185 (32.40)	109 (36.33)	
High	188 (32.92)	98 (32.67)	
Family History of ESCC, N (%)			
Yes	41 (7.18)	78 (26.00)	<0.001
No	530 (92.82)	222 (74.00)	
BMI Categories[*], N (%)			
Normal	202 (35.38)	142 (47.33)	<0.001
Underweight	25 (4.38)	85 (28.33)	
Overweight	151 (26.44)	48 (16.00)	
Obese	193 (33.80)	25 (8.33)	
Tobacco Use, N (%)			
Never	432 (75.92)	196 (65.55)	0.008
Current Smoker	47 (8.26)	30 (10.03)	
Former Smoker	51 (8.96)	37 (12.37)	
Nass/Hookah User	39 (6.85)	36 (12.04)	
Opium Use, N (%)			
No	463 (81.37)	208 (69.80)	<0.001
Yes	106 (18.63)	90 (30.20)	
Tea Temperature			
Warm or lukewarm	394 (69.37)	127 (42.62)	<0.001
Hot	155 (27.29)	108 (36.24)	
Very hot	19 (3.35)	63 (21.14)	

	Control, (N=571)	Case, (N=300)	P-value
DMFT Categories [†] , N (%)			
<16	88 (15.86)	29 (9.80)	0.020
16–22	84 (15.14)	36 (12.16)	
23–27	70 (12.61)	32 (10.81)	
28–31	84 (15.14)	56 (18.92)	
32	229 (41.26)	143 (48.31)	
Eating Discomfort Frequency, N (%)			
Rarely	267 (47.09)	145 (48.82)	0.030
Often	111 (19.58)	38 (12.79)	
Most of the time	189 (33.33)	114 (38.38)	
Vegetable Intake (grams/day), mean (SD)	204.96 (133.58)	193.28 (149.56)	0.240

* BMI: Body Mass Index.

[†] DMFT: Decayed, Missed and/or Filled Teeth

Table 2

Red meat, poultry and fish intake and cooking practices, drinking water source, and esophageal squamous cell carcinoma (ESCC) in the Golestan Case-Control Study in Golestan, Iran *

	Control, (N=571)	Case, (N=300)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Food Related Exposures [†]				
Red Meat Intake (grams/day), N (%)				
<107	151 (27.16)	47 (19.92)	1	1
108–175	154 (27.70)	44 (18.64)	1.35 (0.88, 2.09)	0.86 (0.38, 1.95)
176–268	134 (24.10)	64 (27.12)	1.27 (0.83, 1.94)	2.13 (0.89, 5.11)
>269	117 (21.04)	81 (34.32)	1.25 (0.82, 1.92)	2.82 (1.21, 6.57) **
Poultry, N (%)				
No	125 (21.89)	114 (38.00)	1	1
Yes	446 (78.11)	186 (62.00)	0.39 (0.29, 0.53)	0.67 (0.36, 1.26)
Fish, N (%)				
No	160 (28.02)	170 (56.67)	1	1
Yes	411 (71.98)	130 (43.33)	0.42 (0.30, 0.59)	0.32 (0.14, 0.74)
Fried Meat, N (%)				
No	139 (24.42)	33 (11.04)	1	1
Yes	430 (75.57)	266 (88.96)	2.83 (1.83, 4.39)	3.34 (1.32, 8.45)
Fried Fish, N (%)				
No	325 (57.12)	149 (49.83)	1	1
Yes	244 (42.88)	150 (50.17)	2.73 (1.75, 4.27)	2.62 (1.24, 5.55)
Drinking Water Source				
Drinking Water Source, N (%)				
Piped	516 (90.53)	224 (74.67)	1	1
Others	54 (9.47)	76 (25.33)	4.28 (2.72, 6.74)	4.25 (2.23, 8.11)
Years Using Unpiped Water, mean (SD) ^{††}	44.26 (15.20)	48.59 (16.68)	1.51 (1.31, 1.74)	1.47 (1.22, 1.78)

* Variables included in the model: ethnicity, education, wealth score, opium use, tobacco smoking, family history of ESCC, BMI categories, tea drinking temperature, vegetable intake (amount), DMFT categories and eating discomfort frequency.

[†] Analyses were restricted to those reported eating red meat, poultry or fish.

** P-value for trend <0.05.

^{††} ORs were calculated for each 10 years using unpiped water.