

Food preparation methods, drinking water source, and esophageal squamous cell carcinoma in the high-risk area of Golestan, Northeast Iran

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Cooking practices and water sources have been associated with an increased risk of cancer, mainly through exposure to carcinogens such as heterocyclic amines, polycyclic aromatic hydrocarbons, and nitrates. Using data from the Golestan case-control study, carried out 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 was gathered from 300 cases and 571 controls matched individually for age, sex, and neighborhood using a structured questionnaire and a semiquantitative food frequency questionnaire. Multivariate conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) adjusted for potential confounders and other known risk factors including socioeconomic status and smoking. More than 95% of the participants reported eating meat, mostly red meat. Red meat consumption above the 75th percentile increased the odds of ESCC by 2.82-fold (95% CI: 1.21–6.57). Fish intake was associated with a significant 68% decrease in ESCC odds (26%, 86%). Among meat eaters, ORs (95% CI) for frying meat (red or white) and fish were 3.34 (1.32–8.45) and 2.62 (1.24–5.5). Drinking unpiped water increased ESCC odds by 4.25 times (2.23–8.11).

Introduction

The so-called Asian Esophageal Cancer Belt, which stretches from north-central China to northeastern Iran, houses some of the highest rates of esophageal squamous cell carcinoma (ESCC), the most common type of esophageal cancer (Jemal *et al.*, 2011). 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 minimal effects in these populations (Islami *et al.*, 2004; Tran *et al.*, 2005; Nasrollahzadeh *et al.*, 2008). Unlike most parts of the world, where ESCC is most commonly found in men, the male : female ratio is almost one in areas that have the

The OR for each 10-year increase in the duration of drinking unpiped water was 1.47 (1.22–1.78). Our results suggest roles for red meat intake, drinking water source, and food preparation methods in ESCC, even after adjusting for a large number of potential confounders. *European Journal of Cancer Prevention* 25:123–129 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

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highest incidence rates of ESCC of the world, that is, the Golestan Province of Iran and Taihang Mountain Regions in China (Mahboubi *et al.*, 1973; Yang, 1980; Kamangar *et al.*, 2007). The similarity in incidence rates of ESCC in both sexes implies a role for exposures affecting both sexes equally in the etiology of ESCC (Islami *et al.*, 2009b; Jemal *et al.*, 2011).

A role for diet in the etiology of ESCC in high-risk areas has been implicated since the early 1970s (Kmet and Mahboubi, 1972; Hormozdiari *et al.*, 1975; Yang, 1980; Manchanda *et al.*, 1987). Although the association between low intake of fresh fruits and vegetables and

ESCC has been supported consistently by epidemiologic studies, evidence suggesting a role for animal-based foods (red meat, poultry, and fish) in the etiology of ESCC is limited (American Institute for World Cancer Research Fund/Cancer Research, 2007; Islami *et al.*, 2009a; Kamangar *et al.*, 2009). 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 (Yang, 1980; Manchanda *et al.*, 1987; Skog, 1993). Food preparation practices are among the possible mechanisms through which dietary habits could increase the risk of ESCC (Skog, 1993). 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 (Skog, 1993; Destefani *et al.*, 1997; Sinha *et al.*, 2000; Cross *et al.*, 2007; IARC, 2010). Ingestion of HCAs and PAHs has been shown to induce gastrointestinal (GI) tract tumors in animal models (Hoogervorst *et al.*, 2003; Sugimura *et al.*, 2004). The increased risk of ESCC associated with red meat intake has been attributed partially to PAH and HCA exposure during cooking in previous studies (Perello *et al.*, 2009; IARC, 2010).

The source of drinking water has been implicated consistently in the incidence and mortality of ESCC in high-risk areas of Iran and China (Yang 1980; Tran *et al.*, 2005; Islami *et al.*, 2009b). 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 (Yang *et al.*, 1999; Wu and Li, 2007; Keshavarzi *et al.*, 2012).

Accurate estimation of the intake of such carcinogens is challenging in epidemiologic studies. Assessment of contact with 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, carried out 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 (Nasrollahzadeh *et al.*, 2008). 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 undertake esophagogastroscopy. Biopsy samples obtained during the procedure were reviewed by expert pathologists at the Digestive Disease Research Institute

of Tehran University of Medical Sciences. Histologically proven ESCC cases who were older than 18 years of age, 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 matched individually for age (± 2 years) and sex were enrolled. A family health census was used to develop a roster of eligible controls for each case both in rural and in urban areas. In urban areas, 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 individual could not participate, the second random individual on the list was approached. Approximately 80% of controls were the first randomly selected individuals. The lack of an eligible control was the reason for nonparticipation in the study in almost all the instances, and there was no exclusion because of a lack of consent. For $\sim 10\%$ of cases, only one eligible control could be recruited.

Data collection

Participants were interviewed by trained physicians and nutritionists who could 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. Appliance ownership variables were used to create a wealth score using multiple correspondence analysis and participants were categorized into wealth score tertiles: low, middle, and high. Education and wealth score categories were used as indicators of socioeconomic status (SES) (Islami *et al.*, 2009c).

Weight (kg) and height (cm) were measured to the nearest 0.5 kg and 0.1 cm, respectively. BMI was calculated by dividing measured weight (kg) by the square of the measured height (m), and categorized using the WHO cut-offs: 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 ($\text{BMI} \geq 30 \text{ kg/m}^2$) (WHO, 1995).

Lifelong history of tobacco and opium use was assessed separately. Information on the starting and quitting age and the amount used each time was 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 the following groups: never smokers, former cigarette smokers, current cigarette smokers, and those who smoked other forms of

tobacco (nass, hookah, or a pipe). Similarly, 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 drank it. Tea drinking temperature was categorized into warm, lukewarm, hot, or very hot (Islami *et al.*, 2009d).

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 four 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 previous source of drinking water was also determined 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) developed specifically for this population (Malekshah *et al.*, 2006). 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. The daily intake of each item was calculated by multiplying the consumption frequency by the typical portion size and the number of servings. The total daily consumption of red meat, poultry, fish, and vegetables was calculated by summing 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 g/day). Poultry and fish intake was low and there was not much variation in the amounts reported. Thus, we categorized participants into consumers and nonconsumers of poultry or fish. Processed meat/fish included any meat or fish that had been subjected to preservation methods other than freezing such as salting (with or without nitrites), smoking, air drying, or heating. As processed meat/fish intake was very low 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 Digestive Disease Research Institute of Tehran University of Medical Sciences, the US National Cancer Institute, and the WHO's International Agency for Research on Cancer, and all participants provided written informed consent before enrollment.

Statistical analysis

Normally and non-normally distributed continuous variables are presented as means (\pm SD) and median (interquartile range). Student's *t*-test and the nonparametric Wilcoxon rank sum test were used to test the 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 χ^2 -test.

We used conditional logistic regression to calculate odds ratios (OR) and their corresponding 95% confidence intervals (CI) for food-related and water-related exposures. The associations between frying fish and meat (red or white) and ESCC were only studied among those consuming fish or meat, respectively. Individuals reporting no intake of red meat, poultry, or fish were excluded from this analysis.

We found no evidence of clear multicollinearity as the variance inflation factors were all below 1.5. Separate multivariate models were then fitted to assess the association between food-related 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. As alcohol drinking is extremely uncommon in this area, we did not include alcohol in our analysis (Islami *et al.*, 2004; Kamangar *et al.*, 2007; Islami *et al.*, 2009b). All tests of hypothesis were carried out at a confidence level of 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 years. 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 from urban areas 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

Table 1 Characteristics of cases and controls in the Golestan case-control study of esophageal squamous cell carcinoma 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 (%)]			0.018
Turkmen	312 (54.64)	171 (57.00)	
Non-Turkmen	259 (45.36)	129 (43.00)	
Place of residence [N (%)]			0.700
Urban	150 (26.27)	82 (27.33)	
Rural	421 (73.73)	218 (72.67)	
Education [N (%)]			0.040
None	474 (83.01)	267 (89.00)	
Primary school	64 (11.21)	25 (8.33)	
Middle school or above	33 (5.78)	8 (2.67)	
Wealth score [N (%)]			0.400
Low	198 (34.68)	93 (31.00)	
Middle	185 (32.40)	109 (36.33)	
High	188 (32.92)	98 (32.67)	
Family history of ESCC [N (%)]			<0.001
Yes	41 (7.18)	78 (26.00)	
No	530 (92.82)	222 (74.00)	
BMI categories [N (%)]			<0.001
Normal	202 (35.38)	142 (47.33)	
Underweight	25 (4.38)	85 (28.33)	
Overweight	151 (26.44)	48 (16.00)	
Obese	193 (33.80)	25 (8.33)	
Tobacco use [N (%)]			0.008
Never	432 (75.92)	196 (65.55)	
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 (%)]			<0.001
No	463 (81.37)	208 (69.80)	
Yes	106 (18.63)	90 (30.20)	
Tea temperature			<0.001
Warm or lukewarm	394 (69.37)	127 (42.62)	
Hot	155 (27.29)	108 (36.24)	
Very hot	19 (3.35)	63 (21.14)	
DMFT categories [N (%)]			0.020
< 16	88 (15.86)	29 (9.80)	
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 (%)]			0.030
Rarely	267 (47.09)	145 (48.82)	
Often	111 (19.58)	38 (12.79)	
Most of the time	189 (33.33)	114 (38.38)	
Vegetable intake [mean (SD)] (g/day)	204.96 (133.58)	193.28 (149.56)	0.240

DMFT, decayed, missed, and/or filled teeth; ESCC, esophageal squamous cell carcinoma.

consumption was similar in cases and controls [205 g/day (133.6) vs. 193.3 g/day (149.6); $P=0.2$].

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 rank sum test <0.001). The median (interquartile range) daily consumption of red meat was 15.2 g/day (4.3–35.4) in ESCC cases and 8.5 g/day (2.8–21.0) in controls, respectively (P -value <0.001). In the adjusted model, compared with red meat consumption below the 25th percentile (quartile 1), red meat consumption above the 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% nonsignificant and a 68% significant decrease in the odds of ESCC associated with the consumption of poultry and fish, respectively (Table 2). Our results suggest that, compared with those eating fish that is not fried, both not eating fish and eating deep-fried fish significantly increase the likelihood of ESCC.

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 by 2.6 (1.2–5.6) times in those frying fish, respectively. The crude and adjusted estimated ORs (95% CI) for the meat-related and fish-related exposures are presented in Table 2.

Drinking un piped water was associated with an increased odds of ESCC; we observed a 4.3-fold increase (2.2–8.1) in the odds of ESCC associated with drinking un piped water and a significant 47% increase (22%–78%) in the odds of ESCC associated with every 10-year increase in the duration of using un piped 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 associated inversely with ESCC. Frying both meat and fish was associated with an increased likelihood of ESCC. Finally, ESCC was associated positively with drinking un piped water and the duration of using un piped water.

Red meat intake has been shown to be associated with an increased risk of cancer in different organs including the colon, lung, stomach, and esophagus (Gonzalez *et al.*, 2006; Cross *et al.*, 2007, 2011). Unlike colorectal cancer, the evidence supporting a role for red meat in upper GI cancer is limited (American Institute for World Cancer Research Fund/Cancer Research, 2007). Results of population-based prospective studies in low-risk areas and case-control studies in high-risk areas all consistently show a positive association between red meat intake and ESCC (Cross *et al.*, 2011; De Stefani *et al.*, 2012a). Like other reports, we observed an increase in the odds of ESCC associated with the highest daily consumption of red meat.

The exact mechanism by which red meat is involved in carcinogenesis is not yet well understood. However, the heme iron, saturated fat content of the red meat, and contamination with carcinogenic compounds including

Table 2 Red meat, poultry, and fish intake and cooking practices, drinking water source, and esophageal squamous cell carcinoma in the Golestan case-control study in Golestan, Iran^a

	Control (N=571)	Case (N=300)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Food-related exposures ^b				
Red meat intake (g/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) ^c
Poultry [N (%)]				
No	216 (37.83)	179 (59.67)	1	1
Yes	355 (62.17)	121 (40.33)	0.39 (0.29–0.53)	0.67 (0.36–1.26)
Fish [N (%)]				
No	125 (21.89)	114 (38.00)	1	1
Yes	446 (78.11)	186 (62.00)	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)] ^d	44.26 (15.20)	48.59 (16.68)	1.51 (1.31–1.74)	1.47 (1.22–1.78)

CI, confidence interval; OR, odds ratio.

^aVariables 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.

^bAnalyses were restricted to those reported eating red meat, poultry, or fish, and individuals reporting no intake of red meat, poultry, or fish were excluded from this analysis ($n = 43$).

^c P -value for trend < 0.05 .

^dORs were calculated for every 10 years of use of unpiped water.

HCAs and PAHs, which are formed during high-temperature cooking, have been suggested to play a role in the carcinogenic process (Berretta *et al.*, 2012; Ward *et al.*, 2012). The individual roles of the proposed mechanisms in ESCC carcinogenicity have not been investigated thoroughly (Cross *et al.*, 2011). A positive association between heme iron intake and adenocarcinoma of the esophagus, but not ESCC, has been reported (Ward *et al.*, 2012).

Poultry and fish consumption have been shown to be associated with a lower risk of cancer. Results of the National Institute of Health AARP Diet and Health study indicated a more than 10% reduction in the risk of ESCC for every 10 g increase in poultry and fish intake (Daniel *et al.*, 2011). A case-control study carried out in Uruguay, a high-risk area for ESCC, also reported a decrease in ESCC odds with increased consumption of poultry and fish (De Stefani *et al.*, 2003). For individual evaluations of poultry and fish, however, the results are conflicting. Although fish intake alone was not associated with the risk of ESCC in the AARP study (Daniel *et al.*, 2011), other studies have reported a strong protective effect for fish intake (De Stefani *et al.*, 2003, 2012a). In our study, only fish intake was significantly associated with a reduction in ESCC odds. The anti-inflammatory properties of ω -3 fatty acids and α -linolenic acids have been proposed as one of the plausible mechanisms for the observed protective effect of fish intake (De Stefani *et al.*, 2012b). 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 (Cross *et al.*, 2003). In our study, adjusting for meat intake strengthened the negative association between fish intake and ESCC (data not shown); thus, this substitution is unlikely to play a significant role in this protective association. Fish and poultry intake may also be a surrogate for 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 the risk of ESCC, 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 (De Stefani *et al.*, 2003, 2012a; Hakami *et al.*, 2014). In a previous study, we observed a higher level of deep frying in individuals from the high-risk area of Golestan (Hakami *et al.*, 2014). Consistent with the results of the case-control study in Uruguay, we observed associations between frying meat and fish and ESCC (De Stefani *et al.*, 2012a). HCA contamination of meat during high-temperature frying has been suggested as a possible mechanistic explanation for this association (Skog 1993; Perello *et al.*, 2009; IARC, 2010). Compared with other cooking methods, some of the highest levels of HCAs are formed during high-temperature frying (Skog, 1993; Perello *et al.*, 2009). The oral ingestion of HCAs has been

shown to induce upper GI cancers in animal studies (Wattenberg *et al.*, 1979). HCA (MeIQx and DiMeIQx) intake was also associated with an increased risk of ESCC in a case-control study in Uruguay (Terry *et al.*, 2003).

PAHs are also believed to play a role in esophageal cancer even among nonsmokers (Etemadi *et al.*, 2012). There is a striking dose-response relationship between the PAH content of nontumoral esophageal biopsies and ESCC (Abedi-Ardekani *et al.*, 2010). 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 (Roth *et al.*, 2001; Kamangar *et al.*, 2005). The level of PAH-related DNA adducts is also very high in Golestan, even among female nonsmokers (Etemadi *et al.*, 2012). Hakami *et al.* (2008) have shown an increased amount of PAH intake from staple food in Golestan, which may contribute to this high exposure. Increased levels of carcinogenic PAH in the kitchen air and meat following frying of meat have been reported elsewhere (IARC, 2010). A positive association between red meat intake and urinary 1-OHPG has also been reported in high-risk areas of Golestan (Islami *et al.*, 2012).

Drinking water is another potential route of exposure to different carcinogens such as nitrates, phosphates, oil, and heavy metals (Ward *et al.*, 2005). Several studies highlight a role for high levels of nitrate in drinking water in the etiology of ESCC, possibly through the formation of *N*-nitroso compounds (Yang 1980). Compared with low-risk areas, drinking water contains more nitrate in the high-risk areas of Golestan (Keshavarzi *et al.*, 2012), consistent with a role for nitrate-contaminated water in the etiology of ESCC. Old uniped water sources, such as wells and cisterns, in this region contain higher amounts of nitrates, even compared with newer piped water sources in the same neighborhood. In their ecological study, Keshavarzi *et al.* (2012) also showed that the contents of nitrates, sulfates, and some minerals in drinking water correlate with the mortality rate from esophageal cancer. The association between uniped water source and the risk of ESCC has also been shown in Linxian, China, another high-risk region for ESCC (Tran *et al.*, 2005). 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 association observed cannot solely be because of SES. In our study, as cases and controls were matched on neighborhood, this association is unlikely to reflect regional variations in the incidence of ESCC. The dose-response relationship between duration of drinking uniped water and ESCC further supports this hypothesis.

Our study has some limitations, some of which are common to all case-control studies. Because of the

retrospective design of this study, we could not 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 individuals to affect the risk of ESCC; thus, it is unlikely that recall affects the association observed. Moreover, ESCC patients are more likely to reduce rather than increase meat intake because of difficulty swallowing; therefore, any potential bias because of the reverse causation is expected to occur in the opposite direction to 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 also could not separate the effect of frying poultry from that of red meat because they were reported together.

Some of the 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 as 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 individuals 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 that could have a significant public health impact.

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Conflicts of interest

There are no conflicts of interest.

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