Research Article

Cancer Prevention Research

A Distinct Epidemiologic Pattern of Precancerous Lesions of Esophageal Squamous Cell Carcinoma in a High-risk Area of Huai'an, Jiangsu Province, China

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Abstract

Better understanding of esophageal precancerous lesions (EPL) can inform prevention strategies for esophageal squamous cell carcinoma (ESCC). Here, a cross-sectional epidemiologic study based on the Early Diagnosis and Early Treatment Project of Esophageal Cancer database from 2011 to 2017 was performed to fully investigate and characterize the epidemiology of EPL in rural Huai'an District. Data of 11,518 participants ages 35–75 years were collected through face-to-face interviews by questionnaire. Participants underwent a routine endoscopy examination, tissues were biopsied, and diagnosed according to the histologic criteria of dysplasia. Unconditional univariate and multivariate logistic regression analyses were performed to obtain crude and adjusted odds ratios and corresponding 95% confidence intervals, respectively. A total of 667 subjects were diagnosed with EPL. Factors associated with an increased risk of EPL included: drinking shallow well water and surface water, irregular diet, excessive smoking, exposure to secondhand smoke, consumption of corn, corn flour, pickled food, fried food, and hot food, and having a history of digestive system diseases. In addition, liquor use, but not other alcohol types, contributed to risk of EPL. Consumption of deep well water and vegetables, fruits, and animal livers were associated with lower EPL risk. This study suggested a completely distinct pattern that alcohol use plays only a minor role in EPL and excessive tobacco use shows a significant association in rural Huai'an District, while eating habits and environmental exposure may be the dominant factors. This work may be promising to provide scientific evidence to support primary prevention of ESCC in this region.

Introduction

Esophageal cancer is the eighth most common cancer and the sixth most common cause of death from cancer at the global level. The 5-year survival rate of approximately 15%–25% is so low due to its extremely aggressive nature and advanced-stage diagnosis (1).

Huai'an District, Huai'an City, is an inland rural area in the Northern Jiangsu Province of China with a population with high risk for esophageal cancer with considerably high morbidity and mortality rates. From 2008 to 2012, the incidence of esophageal cancer among adults above 40 years old in Huai'an District was 208.09/100,000 according to the cancer registry report provided by Center for Disease Control and Prevention (CDC) in Huai'an District. The crude incidence and mortality of esophageal cancer in Huai'an District during 2008 and 2012 were 96.15/100,000 and 63.25/100,000, respectively.

Esophageal cancer can be classified into two histologic subtypes: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). EAC is characterized by development at the junction of esophagus and stomach and has become increasingly common in more highly developed world. ESCC develops in the upper part of esophagus and is the predominant type of esophageal cancer in developing countries, including China (2). There are clear differences in the risk factors associated with the incidence and distribution of both EAC and ESCC (1).

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Tobacco smoking, heavy alcohol consumption, frequent consumption of pickled vegetables and hot foods, and low intake of vegetables and fruits have been found to be risk factors for ESCC in many areas, but many causes of ESCC vary among different regions (3). The reasons for the high prevalence of ESCC in Huai'an are not yet entirely understood.

The recognized precancerous lesion for ESCC is esophageal squamous dysplasia, which often occurs in the esophageal mucosa (2). Esophageal precancerous lesions (EPL) for ESCC can be classified into mild, moderate, and severe dysplasia according to the severity of esophageal epithelial lesion (4), and they are associated with increased risk of ESCC of approximately 3-, 10-, and 30-fold, respectively, when compared with normal (5). The classification of EPL referring to histologic criteria of dysplasia was illustrated previously based on the experience over the past decades (6). Squamous dysplasia requires the presence of nuclear atypia (enlargement, pleomorphism, and hyperchromasia), loss of normal cell polarity, and abnormal tissue maturation without invasion of epithelial cells through the basement membrane. Compared with normal, these abnormalities are confined to the lower third of the epithelium in mild dysplasia, whereas they are present in the lower two-thirds of the epithelium in moderate dysplasia, and they also involve the upper third of the epithelium in severe dysplasia. In addition, carcinoma *in situ*, which is full thickness involvement of the epithelium, is considered synonymous with severe dysplasia and included under this category, due to their similarities in histologic appearance as well as the risk of progression to invasive ESCC (6). Twenty-four percent of mild dysplasia, 50% of moderate dysplasia, and 74% of severe dysplasia were reported to develop ESCC during 3.5-year period (5). Previous studies have analyzed that some influencing factors for EPL may be similar to those previously identified as influencing factors for ESCC, such as family history of cancer, tooth loss, and water source (6). However, most current studies have been focused on ESCC, while large population–based EPL researches have rarely been studied.

Although radical esophagectomy can be applied to treat esophageal cancer and severe dysplasia, the postoperative mortality and morbidity remain high due to neoplastic recurrence or incomplete resections (7). In some areas where the incidence of ESCC is high, early diagnosis in the high-risk population meets the requirements for cost-effectiveness of endoscopy and is frequent, thus has promoted the prevention and definitive treatment and achieved very effective results (1). It is therefore of considerable interest that early detection of ESCC by performing endoscopic screenings and the development and establishment of therapeutic endoscopic techniques have been more important (7).

Hence, the Early Diagnosis and Early Treatment Project of Esophageal Cancer (EDETPEC), which is supported by the government and Cancer Foundation of China, has been conducted in Huai'an since 2010 to improve the prevention of ESCC. Our previous etiologic study indicated that dietary and environmental exposures, some demographic parameters, and genetic polymorphism may play important roles in the esophageal carcinogenesis in this endemic region, such as consuming moldy food, pickled vegetables, eating fast, introverted personality, passive smoking, as well as esophageal lesion were recognized as risk factors (8). We realized that it is essential to establish cost-effective prevention strategies, thus EPL has always been of concern to our research group. This population-based cross-sectional epidemiologic study aimed to fully and further investigate the association between these factors and risk of EPL including mild, moderate, and severe dysplasia and characterize the epidemiology of EPL in Huai'an District over the past 7 years, so as to further study the development of ESCC in this specific region and provide scientific evidence to support primary prevention of ESCC.

Materials and Methods

Study population

Village medical staffs were responsible for contacting with each invited participant in each village for recruitment, and the rate of compliance reached approximately 80% in total. Between January 2011 and December 2017, 11,655 residents (4,500 men and 7,155 women) aged from 35 to 75 years old were recruited in the EDETPEC in Huai'an District, Huai'an City, Jiangsu province (study site is shown in Fig. 1). Residents with a history of any form of cancer were excluded, leaving 11,518 participants (4,431 men and 7,087 women; 667 EPL cases and 10,851 normal controls) in the study. The study protocol was approved by the Institutional Review Board of Southeast University Zhongda Hospital (Jiangsu Province, China) with approval number 2012ZDllKY19.0, in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant.

Lesion diagnosis

All the participants underwent a routine endoscopy examination. As previously described in a study of endoscopic screening for EPL (9), 10 mL of 1.2% Lugol iodine solution was sprayed on the esophageal mucosa from top to bottom uniformly. Mucosal staining was observed, and normal esophageal mucosa would turn brown (iodine-positive), while dysplastic lesions would remain unstained (iodine-negative), because glycogen depletion in dysplasia cells leads to the low combination with iodine. Subsequently, unstained tissues were sampled, biopsies were oriented on filter paper, placed in 10% phosphate-buffered formalin and transferred to the pathology laboratory. The biopsies were processed to paraffin blocks and then

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Figure 1.
Map of Huai'an City and the study site of Huai'an District, which is circled with arrow.

prepared on the slides. Histopathologic diagnosis was made after biopsies were stained with hematoxylin–eosin. The previously described histologic criteria of dysplasia were used to classify samples as EPL-positive, and 667 EPL (306 men and 361 women) cases were diagnosed finally.

Baseline data collection

After obtaining written informed consent, epidemiologic data were collected by face-to-face interviews using a comprehensive questionnaire. The questionnaire included the collection of detailed information about sociodemographics (including but not limited to gender, age, educational level, annual income, BMI, and blood pressure), lifestyle (including but not limited to smoking, drinking water source, regularity of diet, and grain storage), eating habits, history of digestive diseases (gastroenteritis, gastroduodenal ulcer, esophagitis, and hepatitis), and family history of cancers. Irregular diet was defined as skipping meals or eating meals at irregular time. In addition, details of substance use (tobacco and alcohol) were collected, including the average number of cigarettes smoked per day (1 pack has 20 cigarettes), alcohol units consumed per day (1 unit is 8 g or 10 mL of pure alcohol), duration of smoking/drinking habit (years), age at starting smoking/drinking, and types of alcoholic beverages regularly consumed (e.g., beer, wine, and liquor). Cumulative consumption of tobacco/alcohol (pack-years/unit-years) was also calculated. Dietary intake was estimated by using a validated qualitative food frequency questionnaire (FFQ), which covered 12 specific food types and 45 specific food items that are commonly consumed in this region. To calculate accurate frequency, the questionnaire had to account for the seasonality of some foods, which would affect consumption frequency throughout the year. Participants were therefore asked how often they consumed the foods per week, as well as per month/per year. They were also asked the duration of the period, in months, they would eat this food (e.g., 12 months for year-round food,

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2 months for very seasonal food). Using this data, we could then standardize this into "times per week" averaged out over the course of a single year. The frequency categories were as follows: "never", "less than once per week," "once per week or more but less than three times per week," and "three or more times per week."

Weight and height were collected using a conventional stadiometer and scale following a standardized procedure. Blood pressure was determined using a sphygmomanometer. Body mass index (BMI; a measure of weight in kilograms divided by the square of the height in meters) was calculated on the basis of the anthropometric measurements of weight (kg) and height (m) and then categorized according to the cut-off values for Chinese population recommended by National Health and Family Planning Commission of the People's Republic of China (10), as underweight if <18.5 kg/m^2, normal if between 18.5 kg/m^2 and 23.9 kg/m^2, overweight if between 24.0 kg/m^2 and 27.9 kg/m^2, and obese if $\geq$28.0 kg/m^2.

Statistical analysis

Unconditional univariate and multivariate logistic regression analyses were performed to calculate crude and adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI), respectively. At first, the association between each factor and risk of EPL was estimated without adjustment for the covariates using the univariate logistic regression model, and all the factors including sociodemographic features, lifestyle, eating habits, and history of diseases were involved. To make the tables more concise, crude ORs of statistically insignificant factors will not be shown in the tables. Subsequently, the statistically significant factors would be reported and selected to be further analyzed by conducting multivariate logistic regression analysis with adjustment for the covariates including gender (male/female), age (ordered categorical variable, 3 levels), BMI (normal/underweight/overweight/obese), education level (illiterate/educated), annual income (ordered categorical variable, 4 levels), as well as the number of cigarettes per day (ordered categorical variable, 5 levels) or/and alcohol units consumed per day (ordered categorical variable, 4 levels). These selected confounders were confirmed on the basis of the previous experience and existing knowledge on esophageal cancer and the univariate logistic regression analysis. In addition, models of adjustment for age or gender alone were conducted as they may materially affect the socioeconomic status, duration of smoking/drinking or alcohol units consumed per day. Likewise, tests for linear trend were performed by assigning the median value of each category of detailed data of smoking and alcohol drinking as a continuous variable in the models. Student *t* test was performed to compare the difference between ages of illiterate and educated groups, and annual incomes of groups with different sources of drinking water. All data were double-entered and validated in Epidata version 3.1 and then processed in Microsoft Excel. Analyses were carried out using SPSS Software (version 22.0; SPSS). Figure was created by GraphPad Prism 7. All tests of significance were two-sided, with a *P* value of 0.05 considered statistically significant.

Results

Subjects' characteristics and lifestyle

The distribution of 11,518 study participants (667 EPL cases and 10,851 normal controls) according to selected sociodemographic features, lifestyle, and eating habits is shown in Table 1. The proportions of women were higher in both EPL cases (54.1%) and controls (62.0%) than those of men. There were more proportions of cases than those of controls who reported to be male and over 60 years old. Multivariate logistic regression analysis also shows that females (OR = 0.77; 95% CI, 0.62–0.94) were less likely to develop EPL when compared with males, and individuals with ages from 51 to 60 (OR = 2.17; 95% CI, 1.66–2.85) and over 60 years (OR = 4.44; 95% CI, 3.41–5.79) had a higher risk of EPL when compared with those aged under 51 years. The results of lifestyle shown in Table 1 revealed that significant positive associations were found between irregular diet and risk of EPL (OR = 1.81; 95% CI, 1.30–2.51). Meanwhile, consuming deep well water as the source of drinking water was significantly associated with a decreased risk of EPL (OR = 0.75; 95% CI, 0.63–0.89) when compared with the reference tap water. However, drinking water from shallow wells or surface water (e.g., lake, river, and pond water) showed an adverse effect on risk of EPL (OR = 3.35; 95% CI, 2.08–5.39). In addition, age-adjusted OR showed that socioeconomic status including educational level and annual income are not significant after adjustment for age. The tabular notes (c and d) below Table 1 show that the average age of educated group were younger than illiterate group ($P < 0.001$), and the subjects drinking shallow well water and surface water had the lowest average income ($P < 0.05$).

Tobacco smoking

Table 2 shows the ORs and 95% CIs for EPL according to selected smoking-related variables in study subjects. After adjustment for potential confounders, consuming more than 30 cigarettes per day was significantly associated with a 75% increased risk of EPL ($P = 0.021$), whereas no statistical significance was found when less than 30 cigarettes were smoked per day. The crude OR shows a positive association between duration of smoking and risk of EPL ($P < 0.001$), whereas no statistical significance was observed after adjustment for selected confounders, because age apparently affects the duration of smoking, which was suggested by age-adjusted OR of duration of smoking. Smoking at earlier ages, particularly beginning a habit before the age of 20, was significantly associated with increased risk of EPL (OR = 1.71; 95% CI, 1.19–2.48).

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Table 1. Characteristics and distribution of study subjects and ORs (and 95% CIs) for EPL according to sociodemographic features, lifestyle, and eating habits

	Cases ($n = 667$) *n* (%)	Controls ($n = 10,851$) *n* (%)	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)[a]	*P*	Age-adjusted OR (95% CI)[b]	*P*
Gender								
Male	306 (45.9)	4,125 (38.0)	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Female	361 (54.1)	6,726 (62.0)	**0.72 (0.62–0.85)**	<0.001	**0.77 (0.62–0.94)**	0.011	**0.82 (0.70–0.96)**	0.014
Age at baseline			P_{trend}	<0.001	P_{trend}	<0.001		
<51	75 (11.2)	3,294 (30.4)	1.00 (reference)	-	1.00 (reference)	—	—	—
51-	216 (32.4)	4,153 (38.3)	**2.28 (1.75–2.98)**	<0.001	**2.17 (1.66–2.85)**	<0.001	—	—
61-	376 (56.4)	3,404 (31.4)	**4.85 (3.77–6.25)**	<0.001	**4.44 (3.41–5.79)**	<0.001	—	—
Education[c]								
Illiterate	313 (46.9)	4,247 (39.1)	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Educated	354 (53.1)	6,604 (60.9)	**0.73 (0.62–0.85)**	<0.001	0.86 (0.71–1.04)	0.113	0.94 (0.77–1.13)	0.493
Annual income per capita (RMB)			P_{trend}	0.054	P_{trend}	0.590		0.429
≤5,000	185 (27.7)	2,511 (23.1)	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
5,001-	317 (47.5)	5,384 (49.6)	**0.80 (0.66–0.96)**	0.019	0.89 (0.74–1.08)	0.242	0.86 (0.71–1.04)	0.120
10,001-	106 (15.9)	1,891 (17.4)	**0.76 (0.60–0.97)**	0.029	0.86 (0.67–1.10)	0.232	0.86 (0.67–1.10)	0.229
15,001-	59 (8.8)	1,065 (9.8)	0.75 (0.56–1.02)	0.064	0.94 (0.69–1.28)	0.684	0.94 (0.69–1.27)	0.669
Source of drinking water[d]			P_{trend}	<0.001	P_{trend}	<0.001		<0.001
Tap water	282 (42.3)	3,999 (36.9)	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Deep well water	361 (54.1)	6,742 (62.1)	**0.76 (0.65–0.89)**	0.001	**0.75 (0.63–0.89)**	0.001	**0.74 (0.63–0.87)**	<0.001
Shallow wells and surface water	24 (3.6)	110 (1.0)	**3.09 (1.96–4.89)**	<0.001	**3.35 (2.08–5.39)**	<0.001	**2.37 (1.32–4.27)**	0.004
Regularity of diet								
Regular diet	623 (93.4)	10383 (95.5)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
Irregular diet	44 (6.6)	468 (4.5)	**1.57 (1.14–2.16)**	0.006	**1.81 (1.30–2.51)**	<0.001	**1.87 (1.35–2.59)**	<0.001

[a]Adjusted for gender, age, BMI, education level, annual income, number of cigarettes per day and alcohol units consumed per day, except the specific variable itself.
[b]Adjusted for age only.
[c]Average ages of illiterate and educated subjects were 58.6 and 53.9 years old respectively ($P < 0.001$, Student *t* test).
[d]Average annual incomes per capita (RMB) of subjects who consume tap water, deep water and shallow wells and surface water were 10,942, 8,469, and 7,576, respectively ($P < 0.05$, Student *t* test).

Table 2. ORs (and 95% CIs) for smoking-related variables with EPL

	Case/control	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)[a]	*P*	Age-adjusted OR (95% CI)[b]	*P*
Number of cigarettes per day							
Nonsmoker	446/7,841	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
<11	73/1,242	1.03 (0.80–1.33)	0.801	0.86 (0.66–1.12)	0.258	0.88 (0.68–1.14)	0.335
11-	110/1,418	**1.36 (1.10–1.69)**	0.005	1.04 (0.82–1.31)	0.776	1.12 (0.90–1.39)	0.331
21-	15/196	1.35 (0.79–2.29)	0.276	0.99 (0.57–1.71)	0.971	1.11 (0.65–1.90)	0.714
31-	23/154	**2.63 (1.68–4.11)**	<0.001	**1.75 (1.09–2.80)**	0.021	**2.05 (1.30–3.23)**	0.002
P_{trend}		<0.001		0.117		0.029	
Duration of smoking (years)							
Nonsmoker	446/7,841	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
<20	45/997	0.79 (0.58–1.09)	0.148	0.75 (0.54–1.04)	0.080	0.77 (0.56–1.06)	0.109
20-	90/1,260	1.26 (0.99–1.59)	0.057	1.05 (0.82–1.35)	0.707	1.12 (0.88–1.42)	0.354
35-	75/679	**1.94 (1.50–2.51)**	<0.001	1.15 (0.87–1.53)	0.317	1.27 (0.98–1.66)	0.071
50-	11/74	**2.61 (1.38–4.96)**	0.003	1.29 (0.67–2.49)	0.441	1.43 (0.75–2.72)	0.279
P_{trend}		<0.001		0.252		0.042	
Age at starting smoking (years)							
Nonsmoker	446/7,841	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
25-	149/2,197	1.19 (0.96–1.44)	0.072	0.93 (0.75–1.14)	0.460	0.97 (0.80–1.18)	0.742
20-	33/486	1.19 (0.83–1.72)	0.342	0.89 (0.61–1.31)	0.553	1.00 (0.69–1.44)	0.984
<20	39/327	**2.10 (1.48–2.96)**	<0.001	**1.71 (1.19–2.48)**	0.004	**1.96 (1.38–2.79)**	<0.001
P_{trend}		0.030		0.013		0.011	
Cumulative amount of smoking (pack-years)							
Nonsmoker	446/7,841	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
<20	81/1,556	0.92 (0.72–1.17)	0.475	0.80 (0.62–1.03)	0.080	0.82 (0.64–1.05)	0.115
20-	36/497	1.27 (0.90–1.81)	0.178	1.09 (0.76–1.57)	0.652	1.14 (0.80–1.63)	0.473
30-	36/440	**1.44 (1.01–2.05)**	0.043	1.06 (0.74–1.53)	0.752	1.14 (0.80–1.63)	0.463
40-	68/517	**2.31 (1.77–3.03)**	<0.001	**1.40 (1.03–1.89)**	0.030	**1.56 (1.18–2.06)**	0.002
P_{trend}		<0.001		0.043		0.002	
Family smoking (exposure to secondhand smoking)							
No	326/6,062	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Yes	341/4,789	**1.32 (1.13–1.55)**	<0.001	**1.47 (1.25–1.74)**	<0.001	**1.37 (1.17–1.60)**	<0.001

[a]Adjusted for gender, age, BMI, education level, annual income and alcohol units consumed per day.
[b]Adjusted for age only.

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Among all the participants, having 40 or more pack-years of cumulative amount of smoking was associated with a 40% risk increase as compared with nonsmokers ($P = 0.030$). In addition, exposure to secondhand smoke due to family smoking was positively associated with a 47% increased risk of EPL ($P < 0.001$).

Alcohol drinking

Table 3 shows the effect of alcohol-related variables on the risk of EPL in the study population. There were no identified strong effects for amount, duration of, and age at starting drinking after the adjustment of several selected confounders, including gender, age, BMI, education level, annual income, and number of cigarettes per day. Age- and gender-adjusted ORs suggest that age and gender materially influence the duration of drinking and the amount of alcohol consumed per day, respectively. A significant positive association was found between liquor intake and risk of EPL based on the result of adjusted OR (OR = 3.22; 95% CI, 1.28–8.13). In addition, the joint effects by cumulative amount of smoking and alcohol drinking were assessed, but no significant interaction was observed ($P = 0.088$; Supplementary Table S1).

Dietary intake

Figure 2 shows the effects of dietary intake on the risk of EPL with ORs (and 95% CIs). Foods were classified into eight categories: vegetables and edible fungi, fruits and nuts, cereals and bean food, livers, pickled food, fried food, hot food, and yeast (food made with yeast). Each food or food type listed in the Fig. 2 was analyzed to be statistically significant when conducting univariate logistic regression analysis (results of univariate logistic regression analysis are shown in Supplementary Tables S2 and S3), and some of them remained significant associations after performing adjusted logistic regression model (Fig. 2). Foods significantly associated with reduced risk of EPL included some types of vegetables, edible fungi, fruits, nuts, bean foods, and liver foods. Alternatively, foods significantly associated with increased risk of EPL included onion (Allium fistulosum L or Allium ascalonicum L) and garlic, corn, corn flour, pickled/salted foods, fried foods, and hot foods. Figure 3 shows the association between dietary intake frequency and age group. The younger group (30–50 years old) tended to consume more vegetables and edible fungi, fruits and nuts, bean food, livers, and food made with yeast, whereas older group (51–75 years old) tended to eat more onion and garlic, corn, pickled food, fried food, and hot food.

Table 3. ORs (and 95% CIs) for alcohol-related variables with EPL

	Case/ control	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)[a]	*P*	Age-adjusted OR (95% CI)[b]	*P*	Gender-adjusted OR (95% CI)[c]	*P*
Alcohol units consumed per day									
Nondrinker	525/8,923	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
<4	25/378	1.12 (0.74–1.70)	0.580	1.00 (0.65–1.53)	0.993	1.07 (0.70–1.62)	0.758	1.02 (0.67–1.54)	0.945
4-	65/983	1.12 (0.86–1.47)	0.390	0.87 (0.65–1.17)	0.365	0.99 (0.76–1.30)	0.963	0.95 (0.72–1.26)	0.726
8-	52/567	**1.56 (1.16–2.10)**	0.003	1.19 (0.86–1.66)	0.295	**1.46 (1.08–1.98)**	0.013	1.29 (0.94–1.77)	0.111
P_{trend}		0.003		0.476		0.029		0.180	
Duration of drinking (years)									
Nondrinker	525/8,923	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
<20	39/639	1.04 (0.74–1.45)	0.830	0.99 (0.70–1.40)	0.956	1.09 (0.78–1.53)	0.625	0.91 (0.65–1.28)	0.590
20-	61/922	1.12 (0.86–1.48)	0.401	0.94 (0.69–1.26)	0.669	1.10 (0.83–1.45)	0.514	0.95 (0.71–1.27)	0.734
35-	42/367	**1.95 (1.40–2.71)**	<0.001	1.08 (0.75–1.55)	0.680	1.29 (0.92–1.80)	0.142	**1.62 (1.15–2.30)**	0.006
P_{trend}		0.001		0.930		0.124		0.088	
Age at starting drinking (years)									
Nondrinker	525/8,923	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
25-	101/1,436	1.20 (0.96–1.49)	0.112	0.93 (0.72–1.18)	0.531	1.05 (0.84–1.31)	0.674	1.25 (0.74–2.12)	0.409
20-	25/311	1.37 (0.90–2.07)	0.142	1.11 (0.71–1.73)	0.643	1.33 (0.87–2.02)	0.191	1.13 (0.74–1.74)	0.566
<20	16/181	1.50 (0.89–2.52)	0.124	1.42 (0.83–2.46)	0.205	1.73 (1.02–2.92)	0.043	1.02 (0.81–1.30)	0.843
P_{trend}		0.361		0.466		0.091		0.338	
Cumulative amount of drinking (unit-years)									
Nondrinker	525/8,923	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
<40	23/333	1.17 (0.76–1.81)	0.467	1.12 (0.72–1.73)	0.627	1.18 (0.77–1.83)	0.450	1.07 (0.69–1.65)	0.772
40-	16/307	0.89 (0.53–1.48)	0.641	0.80 (0.48–1.36)	0.410	0.90 (0.54–1.51)	0.691	0.77 (0.46–1.29)	0.312
80-	15/292	0.87 (0.52–1.48)	0.613	0.73 (0.43–1.26)	0.255	0.82 (0.49–1.40)	0.473	0.74 (0.44–1.27)	0.276
120-	88/996	**1.50 (1.19–1.90)**	0.001	1.07 (0.82–1.40)	0.626	**1.28 (1.01–1.62)**	0.043	1.25 (0.97–1.62)	0.089
P_{trend}		0.002		0.608		0.067		0.122	
Liquor	145/1,887	**1.32 (1.09–1.60)**	0.004	**3.22 (1.28–8.13)**	0.013	1.20 (0.99–1.46)	0.062	1.13 (0.92–1.40)	0.252
Beer	13/282	0.75 (0.43–1.31)	0.304	0.69 (0.38–1.25)	0.220	0.83 (0.47–1.46)	0.513	0.63 (0.36–1.11)	0.111
Wine	5/33	2.48 (0.96–6.36)	0.060	2.13 (0.78–5.82)	0.140	1.96 (0.75–5.11)	0.167	2.47 (0.96–6.36)	0.061

[a]Adjusted for gender, age, BMI, education level, annual income and number of cigarettes per day. Liquor, beer and wine were also adjusted for alcohol units consumed per day.
[b]Adjusted for age only.
[c]Adjusted for gender only.

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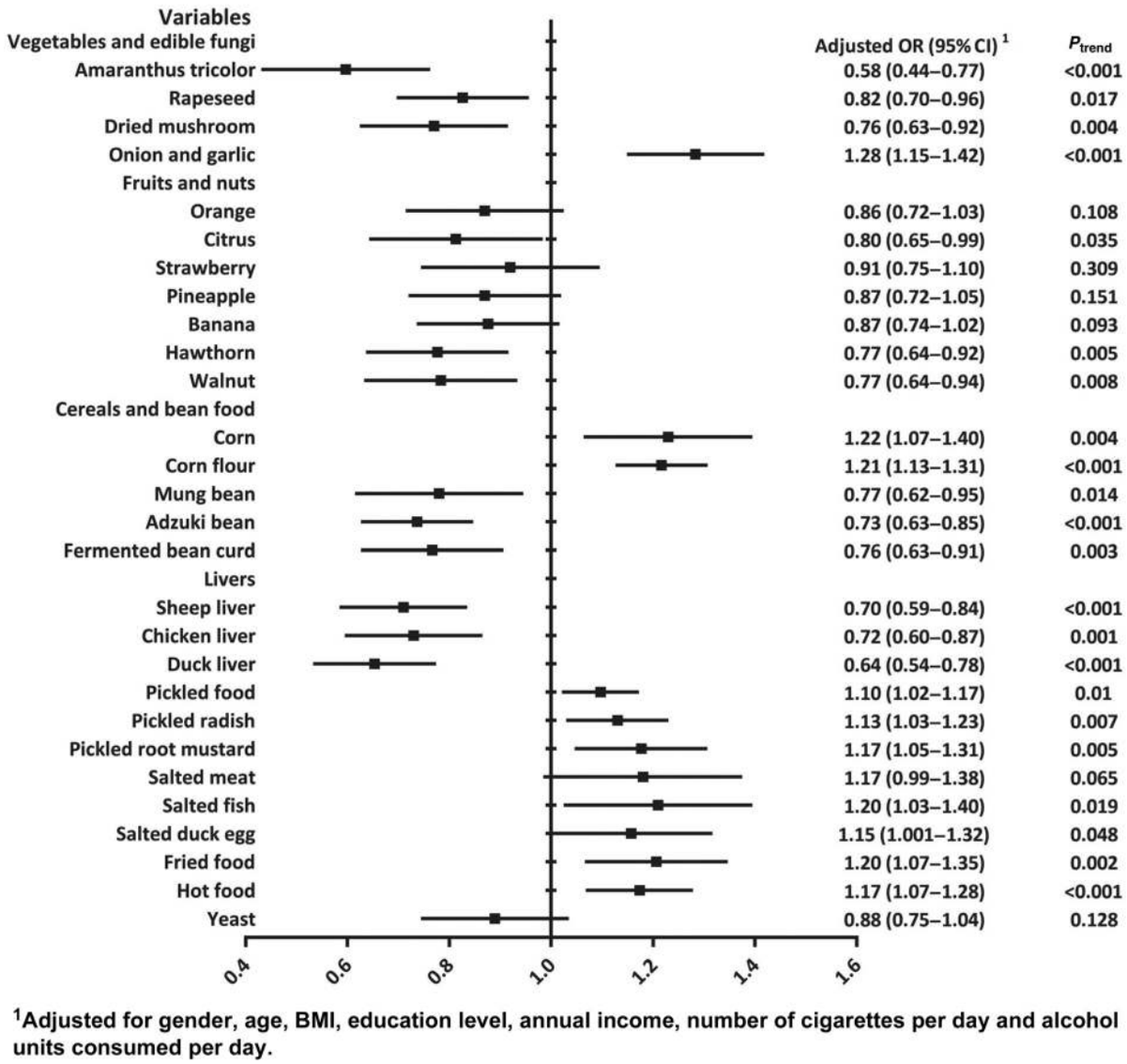

Figure 2.
Association between dietary intake frequency and risk of EPL in study population.

Digestive system disease

Table 4 shows the association between variables of digestive system diseases and the risk of EPL with ORs (and 95% CIs). Having a history of digestive system diseases was positively associated with the risk of EPL after adjustment for potential confounders (OR = 1.35; 95% CI, 1.15–1.59). For example, participants who had ever suffered from gastroenteritis and esophagitis were reported to have increased risk (28% and 77%, respectively). Furthermore, positive association was found between suffering from esophagitis (OR = 2.07; 95% CI, 1.27–3.38) or gastroenteritis (OR = 1.33; 95% CI, 1.04–1.69) after the age of 50 and risk of EPL.

Discussion

Several studies have explored the risk factors influencing esophageal cancer, but risk factors influencing EPL is still largely understudied. With the inclusion of 11,518 participants (667 cases of EPL and 10,851 normal controls), this is probably the largest and most comprehensive research study to date evaluating the association between potential influencing factors and risk of EPL. The association between sociodemographic features, lifestyle, eating habits, history of diseases, and EPL risk were studied. A clear epidemiologic pattern was observed here as alcohol use plays only a minor role in EPL. Eating habits and

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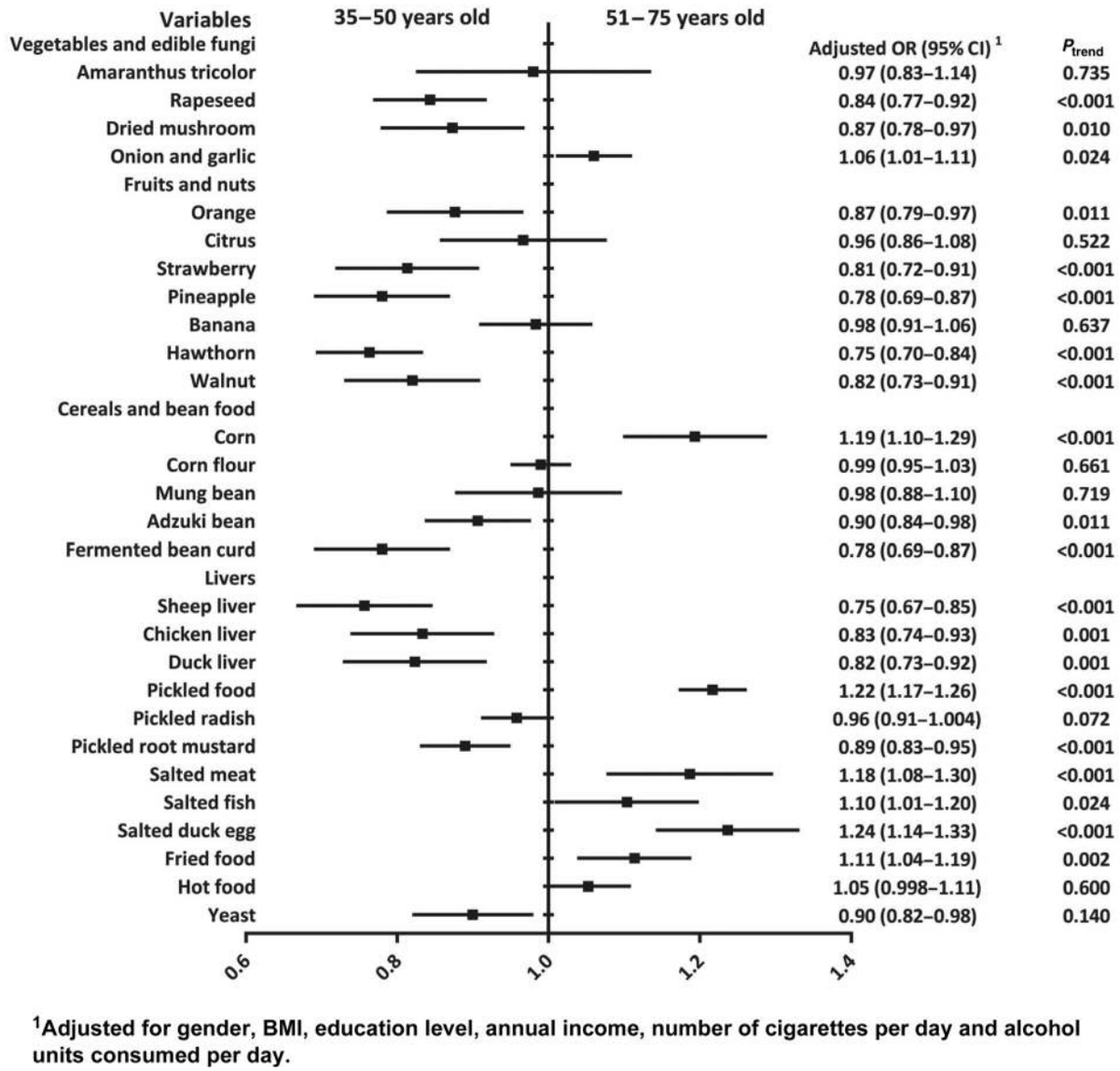

Figure 3.
Association between dietary intake frequency and age groups in study population.

Table 4. ORs (and 95% CIs) for digestive system disease-related variables with EPL

	Case/control	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)[a]	*P*
History of digestive system diseases		**1.32 (1.13–1.55)**	0.001	**1.35 (1.15–1.59)**	<0.001
History of gastroenteritis		**1.26 (1.06–1.49)**	0.010	**1.28 (1.07–1.52)**	0.007
Age of suffering from gastroenteritis					
Disease-free control	490/8,387	1.00 (reference)	—	1.00 (reference)	—
<50	88/1,608	0.94 (0.72–1.18)	0.583	1.21 (0.95–1.53)	0.124
50-	89/856	**1.78 (1.41–2.26)**	<0.001	**1.33 (1.04–1.69)**	0.021
P_{trend}		<0.001		0.033	
History of esophagitis		**1.79 (1.22–2.63)**	0.003	**1.77 (1.19–2.62)**	0.005
Age of suffering from esophagitis					
Disease-free control	638/10,584	1.00 (reference)	—	1.00 (reference)	—
<50	9/150	1.00 (0.51–1.96)	0.989	1.36 (0.68–2.71)	0.383
50-	20/117	**2.84 (1.75–4.59)**	<0.001	**2.07 (1.27–3.38)**	0.004
P_{trend}		<0.001		0.011	

[a]Adjusted for gender, age, BMI, education level, annual income, number of cigarettes per day, and alcohol units consumed per day.

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environmental exposure may be the dominant factors for EPL in Huai'an District.

Sociodemographics

Like esophageal cancer, EPL is more frequently described in men than in women. Generally, men are more likely to have unhealthy lifestyles and therefore more likely to be exposed to risk factors like tobacco and alcohol use (3). Likewise, a significant positive association between age and risk of EPL is observed. This is consistent with previous studies that suggested age as the greatest risk factor for developing esophageal cancer (11), as older people tend to be exposed to risk factors for a longer time and may have reduced immune response against tumors.

Inverse dose–response association between risk of ESCC and socioeconomic status including educational level and annual income was reported previously (12). However, such statistical significance was not observed in our adjusted results of socioeconomic status, and age obviously affects the results of socioeconomic status. The tabular note (c) below Table 1 also shows that the average age of educated group was significantly lower than illiterate group. The reason could be that the lack of education resources became one of the greatest challenges faced by the Chinese education system several decades ago, especially in rural areas. It caused schooling of many Chinese individuals to be delayed or cut short, with long-term consequences for occupational attainment and earnings. Since the reform and opening up, Chinese education system has been geared toward economic modernization. Therefore, younger individuals tend to have higher educational level and annual income but lower risk of EPL. In addition, Huai'an rural District is generally lower middle class without much variation, thus internal comparison within this area may not exhibit dramatic difference in socioeconomic status. Similar results were also observed by a study on risk factors for EPL in Linzhou, a high ESCC risk region of China (13).

Lifestyle

Irregular diet was associated with increased risk of EPL in all participants in our study. Similar results were reported in the previous studies on esophageal cancer (14, 15). However, no previous studies have addressed the direct association between meal irregularity and risk of EPL. Researchers are not yet aware of whether irregular diet changes the mucosal membrane. A previous study hypothesized that the digestive system produces secretions, free radical scavengers, or perhaps some other yet to be discovered chemical so as to be ready to receive food during the usual meal timing, but the secretions or lack of secretions somehow cause the lining of digestive tract to be susceptible to inflammation if no food is ingested during this time (16). Consuming deep well water as the source of drinking water was significantly associated with a decreased risk when compared with the reference tap water, whereas shallow well water and surface water (e.g., lake, river, and pond water) increased the risk of EPL. In Huai'an District, residents had both fee-based tap water and deep well water supplies in their residence, and they could also use the no-charge surface water or shallow well water outside the house. Notes in Table 1 show that the small number in shallow well water and surface water group may be a proxy of the low-income population. Furthermore, the quality of water may play an important role in the development of esophageal cancer, and nitrate in drinking water may be a significant factor (17). Previous study found that the concentrations of methyl ethylamine, morpholine, N-methylbenzylamine, nitrate, and chloride in water from rivers are higher than those in well (18). A significant inverse correlation was detected between esophageal cancer mortality and buffer distance from rivers and lakes within the 20-km distance threshold, meanwhile many regions along Huaihe river drainage basins exhibited high risk of esophageal cancer, and Huai'an is also included (17). Erhe River flowing from Hongze Lake (Hongze Lake can be found in Fig. 1) is the major source water in Huai'an District, but a severe nitrosamine contamination in Erhe River with 312 ng/L N-nitrosomorpholine and 385 ng/L total nitrosamine was reported (19). In addition, algal toxins like microcystin have received much concern as they might be responsible for the development of ESCC when eutrophication occurs in Hongze Lake (20, 21). On the contrary, consuming deep well water as the source of drinking water can be a protective factor of esophageal cancer was reported (22). Interestingly, researchers found that drinking water wells in the regions of high esophageal cancer incidence are generally shallower and have higher nitrate concentrations, as shallow wells are susceptible to contamination via both direct leaching of pollutants into underground water and the hydrological cycle through connectivity with contaminated surface water, whereas low esophageal cancer incidence region has particularly deep wells with extremely low nitrate concentration and high selenium concentration (23, 24).

Strong evidence for a causal association between tobacco smoking and risk of esophageal cancer has been reported previously (3). However, a study illustrated that smoking is a risk for ESCC but not for EPL, although many risk factors studied were concordant for both EPL and ESCC (6). Our adjusted results suggest that excessive tobacco use may cause qualitative changes, so as to contribute to the development of EPL in this region. Adjustment for age materially affects the duration of smoking because older people tend to have a long-term smoking history. In addition, exposure to secondhand smoke was strongly associated with increased risk of EPL according to the adjusted result. Previous study of ESCC showed similar results (25), but relative data of EPL has not been reported before. The region in Huai'an District where we investigated is rural. Family members often share the same living places where

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they stay together for hours, thus all the relatives who are likely to smoke in the presence of study subjects can be sources of secondhand smoke. Sidestream smoke that goes into ambient air directly from burning tobacco products is the main component of secondhand smoke. Over the years, studies have demonstrated the greater toxicity (about four times) and carcinogenicity (about two to six times) of cigarette sidestream than mainstream smoke (26).

Alcohol use has long been thought to be a risk factor for squamous cell carcinoma of esophagus (3). However, in our study only consumption of liquor was found to be statistically associated with increased EPL risk. No statistically significant result was observed in daily alcohol units consumed, duration of drinking, age of initial drinking, and cumulative amount of drinking; hence, no clear association between alcohol use and EPL was found in Huai'an District. Similar result of straight liquor was confirmed in the early study (27), likewise Chinese liquor with 40%–60% alcohol concentration is a kind of straight liquor as drinkers consume it without any mixers or ice. Adjustments for gender and age materially affect the results of daily alcohol units consumed and duration of drinking, respectively, because men are likely to drink far more frequently than women in China and older people tend to have a long-term drinking history. Previous studies of esophageal cancer conducted in the rest, high-risk areas, of Northern Jiangsu Province did not observe any association between alcohol use and esophageal cancer as well (28, 29), suggesting that alcohol exposure plays only a minor role in the development of esophageal cancer in the inland regions of Northern Jiangsu Province including Huai'an. It was also reported that tobacco and heavy alcohol exposure in some high-risk areas are not as strong risk factors as in low-risk areas (3). Furthermore, a completely distinct epidemiologic pattern has been observed in certain high-risk areas located in Taihang mountain region of north-central China as well, where the incidence rates in females are almost as high as males in spite of the fact that women are very rarely exposed to tobacco and alcohol use; thus, tobacco smoking and alcohol drinking having little or no role in this extremely high ESCC risk area was concluded on the basis of their results (30, 31). In Taihang mountain region, the incidence rate of ESCC is only up to about 1.5-fold more common in men than in women (32), which is similar with the data provided by CDC in Huai'an District (94.87/100,000 for male and 78.70/100,000 for female from 2003 to 2006; 95.24/100,000 for male and 63.18/100,000 for female in 2014), whereas ESCC is 2 to 8 times more common in men than in women in most areas of the world (33). In summary, the role of some other factors that may be involved in the development of EPL or carcinogenesis of ESCC are supposed to take the main responsibility in these areas including Huai'an District, such as environmental exposures like drinking water quality, which are discussed above, and dietary factors, as these common factors are likely to influence both sexes equally. Therefore, the risk factors for ESCC and EPL in Huai'an District might be different from those seen elsewhere and result in a completely distinct epidemiologic pattern.

Eating habits

Intake of fresh fruit and vegetables has long been considered to be inversely associated with risk of ESCC (3). Our analysis included most fresh fruit and vegetables that were commonly consumed in Huai'an District, and the final adjusted results show that high intake of hawthorn and nonstarchy vegetables such as amaranthus tricolor and rapeseed is statistically associated with reduced risk of EPL, whereas intake of most kinds of fruit shows a weak protective effect. Antioxidant micronutrients like vitamins and minerals were considered to confer a possible anticancer activity on fruit and vegetables (3), but very few published papers studied the association between intake of fruit and vegetables and risk of EPL.

Figure 2 also shows that increased consumption of dried mushroom, walnut, mung bean, adzudi bean, fermented bean curd, and animal liver can be inversely associated with EPL risk. Generally, the possible mechanism of consumption of these foods against ESCC has long been considered to be the potential immunomodulatory, anticarcinogenic, antimetastasis, and antimutagenic properties provided by high content of antioxidant micronutrients and some bioactive compounds, such as fiber, vitamin A, C, E, B vitamins, selenium, polyphenols, phytosterols, flavonoids, and isoflavones (34–37). The observed protective effect of intake of animal livers is probably because livers are rich in minerals, vitamin A and B vitamins such as folate (38); meanwhile, folate, vitamin A, and its precursor β-carotene have been demonstrated to play a preventive role in various forms of cancer including ESCC (36, 37, 39). A study on systematic comparison of phytochemicals and antioxidant capacities of 13 classes of food legumes illustrated that the strongest oxygen radical–absorbing capacity was found in adzuki bean, followed by mung bean, and the second and sixth highest peroxyl radical–scavenging capacities were found in adzuki bean and mung bean, respectively (40). Adzuki bean and mung bean exhibited outstanding antioxidant activity *in vitro* and antiproliferation properties against digestive system cancer lines, and much higher contents of total phenolic and procyanidin were determined in both of them than in the most of the rest of the legumes (40). Fermentation process confers a much stronger antioxidant and antiproliferative activity on soybean foods, because the isoflavone composition changes during fermented bean curd manufacturing, especially the conversion of the isoflavones from glucoside conjugates to the form of aglycone with a higher bioavailability (41).

As shown in the results of dietary factors in our study, positive associations were observed between risk of EPL and intake of onion (Allium fistulosum L or Allium asca-

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lonicum L), garlic, corn, corn flour, fried food, hot food, and pickled/salted food such as pickled radish, pickled root mustard, salted meat, salted fish, and salted duck egg. Generally, allium vegetables, in particular, raw onion and garlic, are considered to have preventive effects on ESCC, but cooked onion and garlic were not addressed (42). In Huai'an District, allium vegetables are used as spices and will experience a period of time of deep-frying or pan-frying before adding all the food materials. These cooking methods may disable the observed efficacy of onion and garlic, because bioactive components such as allicin and other thiosulfinates can be both unstable and reactive under heating (43). Stir-fried onion and garlic also lost the enzymatic ability to form these bioactive components because alliinase is inactivated in cooking (44). Onion and garlic intakes could be simply considered markers of stir-/deep-/pan-frying diet in China, while fried food intake is a well-known risk factor for cancers. As shown in Fig. 3, older residents (51–75 years old) tended to frequently consume fried food and onion and garlic. Generally, locals do not eat onion and garlic intentionally as they are just spices for frying. Therefore, the daily onion and garlic intake can be low, but the fried food can be frequently consumed. Furthermore, acrylamide, which has been found to have neurotoxicity, carcinogenicity, and genotoxicity in experimental animals and cellular system, was also detected at relatively high levels in stir-fried garlic and onion, with, respectively, the second and third highest acrylamide content in the list of 22 stir-fried vegetables (45). Therefore, extremely high frequency of stir-fried garlic and onion consumption in Huai'an population might contribute to a large cumulative amount of acrylamide intake during their lifetime. A previous study conducted in Jiangsu Province also reported the similar results of the association between esophageal cancer risk and onion consumption (29).

Corn and corn flour are the important staple foods in Huai'an District. Basically, corns are dehydrated by exposure to sun after harvest for storage in this region. However, aflatoxins and fumonisins, which are known carcinogens, often develop in stored corn and flours, and therefore result in a health hazard to human beings (3, 23). A significant positive association between esophageal cancer risk and consumption of corn and flour was also reported (23). Our previous studies have confirmed the contamination of the two mycotoxins in corn in Huai'an, and suggested their contributing role in esophageal carcinogenesis (46, 47). Thus, consuming such contaminated corns and porridge made of corn flour as the staple foods can be a risk factor in Huai'an District.

Previous study conducted in rural areas of Henan province, China, reported that consumption of fried food is a risk factor for both ESCC and its precancerous lesions (48). Stir-frying, deep-frying, and pan-frying are common cooking methods practiced in China, involving heating oil to high temperatures. These cooking methods produce carcinogens such as genotoxic heterocyclic amines, benzo[a]pyrene, formaldehyde, and benzene in both emissions and edible parts of food, especially during the frying of protein-rich food (49). Consumption of hot foods and drinks has long been considered as a risk factor as well (3, 31). An early study recorded that intraesophageal temperature increased by 6°C to 12°C when drinking coffee with different sip sizes at temperature of 65°C (50). These hot foods may cause a carcinogenic effect due to the direct contact with esophageal mucosa, and the upper esophagus may be more harmfully affected than the lower esophagus (42). According to the dietary pattern in Huai'an District, many residents have been used to frequently consume hot foods such as their staple food porridge made of corn flour.

Likewise, pickled/salted food, a kind of very popular food in Huai'an, was found to be associated with increased risk of EPL. Similar finding was observed in a previous study of adverse effects of preserved vegetables on EPL (51). It has been summarized that eating pickled foods is very common and may be a main risk factor in high esophageal cancer risk areas of China (3). Contamination of N-nitroso compounds such as N-nitrosamines in pickled foods has been confirmed to be an important mechanism, and other compounds such as Roussin red methyl ester and mycotoxins released by microorganisms are also thought to be responsible for the carcinogenicity exerted by these food items (52). In addition, it can be found from the combination of Fig. 2 and Fig. 3 that the dietary factors on the left-hand side could be a proxy of the younger group (30–50 years old) diet, while the diet on the right is from the older group (51–75 years old). It is reasonable as local older people may have the habit of making pickled food and preserved corn for long-term storage during the period of food shortages, and they were more likely to fry dishes with using onion and garlic as the spices. Compared with them, the life of younger people has changed and become relatively healthier.

Digestive system diseases

In addition, having a history of digestive system diseases such as gastroenteritis and esophagitis was associated with an increased risk of EPL in our study, and the older age of onset shows a significant association according to the results. Previous study, which reported the similar finding, indicated that the mucosal barrier of digestive tract may be injured after suffering from digestive system diseases; hence, the protection against carcinogens is going to become weaker (53). However, it may be hard to clarify the causality between history of digestive system diseases and risk of EPL, because these diseases and EPL are likely to share many risk factors.

Our research involved a large population in this targeted region, and carried out epidemiologic study as a powerful

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tool for examining associations with EPL risk, which were rarely studied. In the analysis, our models were adjusted for many potential confounding factors to limit confounding. However, this study also had limitations. Recall bias was possible with the questionnaires, and the food frequency questionnaire did not collect information of portion size or cooking methods, which may be confounding variables. It should also be pointed out as the limitation of the cross-sectional study in establishing a causal association.

In summary, a distinct epidemiologic pattern was observed in Huai'an District. Alcohol use plays only a minor role in EPL and excessive tobacco use shows a significant association. Eating habit such as frequent intake of pickled food, fried food, hot food, and environmental exposure such as consuming shallow well water or river/lake water as the source of drinking water may be the dominant risk factors for EPL here. In addition, local older adults have a completely different dietary pattern from younger adults and are generally unhealthier. Our study may be promising to provide scientific evidence to support primary prevention of ESCC in this region. Early detection of individuals with EPL for subsequent prevention strategies may substantially contribute to the reduction of ESCC mortality. On the basis of this study, follow-up can be scheduled for further prospective studies to explore more findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The funding unit had no role in the whole study including study design, collection and analysis of data, and composition of the manuscript.

Authors' Contributions

Conception and design: S. Wang, G. Sun
Development of methodology: S. Wang, G. Sun
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Su, C. Miao, G. Song
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Pan, T. Zhang
Writing, review, and/or revision of the manuscript: D. Pan, P.J. Raine, G. Sun
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Pan, M. Su, T. Zhang, C. Miao, L. Fu, L. Yang, G. Song, P.J. Raine
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