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Risk Factors for Gastric Cancer and Related Serological Levels in Fujian, China: Hospital-based Case-control Study

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Abstract

OBJECTIVE To explore the relationships between gastric cancer and serum PG I, PG II, PG I/II ratio, G-17, and *H. pylori* infection, and to investigate dietary and lifestyle risk factors for gastric cancer in Fujian Province, China.

DESIGN A hospital-based, 1:1 matched case-control study.

SETTING Patients with newly-diagnosed gastric cancer were recruited from the Fujian Provincial Hospital and the No.900 Hospital of the Joint Support Force of the Chinese People's Liberation Army between July 2014 and December 2016.

PARTICIPANTS A total of 180 pairs of gastric cancer patients and control subjects were recruited in the study, including 134 (74.4%) male pairs and 46 (25.6%) female pairs.

INVESTIGATION AND ANALYSIS MEASURES

Serological indicators were tested with ELISA kits. Dietary, lifestyle and psychological factors were investigated through face-to-face questionnaire. Relationships between gastric cancer and these influencing factors were examined by Chi-square test and conditional logistic regression.

RESULTS Serum PG II and G-17 levels and *H. pylori* infection rate were higher in gastric cancer patients than in control subjects ($P < 0.05$), while PG I/II ratio was lower in gastric cancer patients ($P < 0.05$). Serum G-17 levels were higher in patients with corpus gastric cancer than in patients with antral gastric cancer ($P < 0.05$). Serum PG II levels were higher in patients with advanced gastric cancer than in patients with early-stage cancer ($P < 0.05$), however PG I/II ratio was lower in patients with advanced stage gastric cancer than in patients with early-stage cancer ($P < 0.05$). Eating hot food (OR= 2.32), eating pickled vegetables (OR = 4.05), and often feel troubled (OR = 2.21) were found to significantly increase the risk of gastric cancer (all $P < 0.05$), while

consuming onion or garlic (OR = 0.35), drinking tea (OR = 0.26), eating fresh fruit (OR = 0.55), and high serum PG I (OR = 0.99) or PG I/II ratio (OR = 0.73) were found to be protective against gastric cancer.

CONCLUSION Study results showed that serum PG, G-17 and *H. pylori* antibodies could be useful indicators for early diagnosis of gastric cancer. Increase in serum G-17 level might indicate the location of gastric cancer. Increase in serum PG II level and decrease in PG I/II ratio might imply the clinical stage. Eating hot food, eating pickled vegetables, and often feel troubled may be risk factors for gastric cancer, while eating fresh fruit, eating onion or garlic, and drinking tea may be protective factors against the disease.

Key words: Gastric cancer, Risk factor, Pepsinogen, Gastrin, *Helicobacter pylori*

Strengths and limitations of study

Fujian Province, high in gastric cancer incidences, is an important research site for exploring the etiologies of gastric cancer.

This study was one of the few studies to use serum indicators as independent variables to analyze the risk factors for gastric cancer.

Strict quality control was conducted in the selection of new cases to ensure comparable results.

However, as a case–control study, recall bias was inevitable and trial studies are required for more accurate results.

Sample size of this study was not large enough, further studies will recruit more subjects.

INTRODUCTION

Gastric cancer is a common malignancy in the gastric mucosa and gastric glands in the digestive tract and accounts for a high proportion of cancer deaths in China, especially in Fujian Province. According to the World Cancer Report published by the World Health Organization in 2018[1], there were 1,033,071 new cases of gastric cancer (accounting for 5.7% of all cancer incidence) and 782,685 deaths from it (accounting for 8.2% of all cancer deaths) worldwide. Gastric cancer has high disease burdens[2], ranking second only to lung cancer in terms of the number of deaths[1]. In China, there were 456,124 new cases of gastric cancer in 2018, accounting for 44.1% of global incidence[3]. In Fujian Province, located in southeast China, gastric cancer accounted for 12.5% of all cancer incidences in 2014[4].Risk factors for gastric cancer such as dietary, lifestyle, and psychological factors are different across China[5-10]. The overall objectives of this study were to explore the relationship between gastric cancer and serum PG Ⅰ, PG Ⅱ, PG Ⅰ/Ⅱ ratio, G-17 and *H.pylori* infection, and to investigate the risk factors for gastric cancer in Fujian.

METHODS

Study design

This was a hospital-based, 1:1 matched case-control study, performed in accordance to the Declaration of Helsinki of the World Medical Association and approved by the Ethics Committee of the Fujian Center for Disease Control and Prevention. Informed consents were obtained from all recruited subjects. Subjects' dietary and lifestyle data were obtained through face-to-face interviews by trained investigators, and blood samples were collected for the test of serum markers.

Case and control groups

From July 2014 to December 2016, patients with newly-diagnosed gastric cancer were recruited from the Fujian Provincial Hospital and the No.900 Hospital of the Joint Support Force of the Chinese People's Liberation Army. Control subjects were recruited simultaneously from the Medical Examination Center at the same hospital. Gastric patients and control subjects were 1:1 matched according to gender and similar age within 3 years. Inclusion criteria for the gastric cancer group were: newly diagnosed of gastric cancer by gastroscopy and pathology, with no medication history of antibiotics, proton pump inhibitors, or H receptor antagonists. Patients with prior surgeries or chemotherapies were also excluded. Inclusion criteria for the control group were: no diagnosis or symptoms of chronic stomach diseases, and no mental retardation or emotional blockages. All subjects had lived in Fujian Province for more than 10 years.

Risk Factor Survey

Data of risk factors were collected by trained investigators through face-to-face interviews with the subjects, using a consolidated questionnaire. The survey contents included general information, dietary habits(i.e., breakfast consumption, meal duration, intake frequencies of onion or garlic, spicy food, hot food, green vegetables, fresh fruit, dairy, pickled vegetables, and fish sauce), lifestyle habits (i.e., smoking, alcohol consumption, and tea consumption), and psychological factors (i.e., personality type, adaptability, feels about life, and interpersonal skills).

Serum sampling

A total of 5 mL of fasting venous blood was collected from each subject, and was centrifuged at 3000 r/min for 10 min to extract the serum, which was then stored at -80℃ for further testing. Serum PG I, PG II, and G-17 levels were tested using ELISA kits from BIOHIT, Finland (batch numbers

of 19PA1505, 21PB1508, and 19GC1506, respectively). Antibodies to *H. pylori* were assessed using ELISA kits from the AI Kang Company of China (lot number 201510087).

Definitions and variables

Dietary intake frequencies were classified as: never or seldom consumption (no more than once per week), occasional consumption (two to six times per week), and regular consumption (seven or more times per week). Smokers were defined as subjects who had smoked more than 100 cigarettes overall. Alcohol consumers were defined as subjects who had been drinking any alcohol at least once a week for more than six months. Tea consumers were defined as subjects who had been drinking any tea at least once a week for more than six months.

According to the calculated cut-off value, an optical density (OD) value of the sample >1.1 × the cut-off value was considered *H. pylori*-positive, while an OD <0.9 × the cut-off value was considered *H. pylori*-negative.

Statistical analysis

Data were inputted by the double-entry method and tested for consistency using Epidata 3.1. The SPSS version 24.0 software package was used for conducting Chi-squared tests on the demographic information, and for conducting the single and multivariate conditional logistic regression analyses on the other information to determine the odds ratio (OR) and 95% confidence interval (CI). Test significance level was set at 0.05.

RESULTS

Demographic information

A total of 180 pairs of gastric cancer patients and control subjects were

recruited in this study, of which 134 pairs (74.4%) were male subjects and 46 pairs (25.6%) were female subjects. The average ages of the cancer group and the control group were 61.0 ± 10.8 and 60.1 ± 10.9 years, respectively. The two groups were not significantly different in age, marital status, education, or labor intensity (all $P \geq 0.05$), however the occupation compositions were different.

Dietary habits, lifestyles and personality traits

As shown in Table 1, there were significant differences in the dietary habits between cases and controls. Lifestyles, such as smoking, alcohol consumption, and tea consumption, of the two groups were also significantly different (Table 2).

Personality types, adaptability, feelings about life, irritability, and interpersonal skills were also significantly different between cases and controls (Table 2).

Serum PG I, PG II, PG I/II ratio and G-17 levels

Serum PG I, PG II, and G-17 levels did not fit normal distributions, nor did the PG I/II ratio; therefore median values were used to indicate central tendencies and the P25 and P75 percentiles were used to indicate dispersion tendencies. Serum PG II and G-17 levels in gastric cancer patients were higher than those in the control subjects ($P < 0.05$), while the PG I/II ratio was lower in the patients ($P < 0.05$); PG I levels were not statistically different between the two groups. The results of the serum markers are summarized in Table 3.

H. pylori infection

As shown in Table 4, 66.67% of gastric cancer patients were *H.*

pylori-positive, which was significantly higher than that of the control group (54.44%, $P < 0.05$). The positive rate of *H. pylori* was higher in male gastric cancer patients than in controls ($P < 0.05$), however no significant differences were found between female cases and controls or among age groups ($P > 0.05$).

Serological parameters with different locations of gastric cancer

Serum G-17 levels were higher in patients with gastric corpus cancer than in patients with gastric antrum cancer ($P < 0.05$), but not significantly different among patients with other tumor locations ($P > 0.05$; Table 5). Serum PG I, PG II levels, and the PG I/II ratio were not statistically different among patients with different tumor locations ($P > 0.05$; Table 5).

Serological parameters with different clinical stages of gastric cancer

As shown in Table 6, serum PG II levels in patients with advanced gastric cancer were higher than in patients with early-stage gastric cancer ($P < 0.05$). However, the PG I/II ratio was lower in patients with advanced cancer than in patients with early-stage disease ($P < 0.05$). Serum PG I and G-17 levels were not significantly different between the two different clinical stages ($P > 0.05$).

Multivariate analysis

Multivariate conditional logistic regression analysis was performed with gastric cancer as the independent variable, and the dietary/lifestyle habits, psychological factors, serum PG I level, serum PG II level, PG I/II ratio, serum G-17 level, and *H. pylori* infection as dependent variables. As shown in Table 7, eating hot food, consumption of pickled vegetables, and

often feel troubled may be risk factors for gastric cancer. However, the consumption of fresh fruit, onion or garlic, drinking tea, and elevated serum PG I levels and PG I/II ratio might be protective factors for gastric cancer. Other factors examined in this study had no statistically significant effect on gastric cancer ($P > 0.05$).

DISCUSSION

Overall, this study has found that gastric cancer was related to several dietary and lifestyle factors, the changes in serum PG I/II ratio and G-17, and the infection rate of *H. pylori*.

Multivariate conditional logistic regression analysis indicated that the consumption of pickled vegetables may be a risk factor for gastric cancer. Pickles are high in salt and may damage the gastric mucosa, reduce gastric acid secretion, and inhibit the synthesis of prostaglandin E, which enhances gastric mucosa resistance[11]. In addition, pickles contain high amount of nitrate and nitrite, which can be converted to N-nitrosamide, a carcinogen, under gastric acid conditions[12]. In an area with high incidence of gastric cancer in northwest China's Gansu Province[13], where people often consume pickled vegetables as substitutions of fresh vegetables in winters, 65%-75% of pickles had detectable levels of nitrite and all had tested positive for nitrate; and the levels of nitrite and nitrate in the gastric juice were significantly higher in people often consume pickled vegetables than in people who seldom consume pickled vegetables. Nitrate and nitrite levels in the gastric juice were associated with the frequency of pickle intake, which in turn was associated with gastric cancer. This study also

found that regular pickle intake (seven or more times per week) increased the risk of gastric cancer (OR = 4.05; 95% CI: 2.01–8.02).

Study results also indicated that tea consumption was a protective factor against gastric cancer. Tie Guanyin, one of the most popular teas in Fujian, is rich in tea polyphenols. Tea polyphenols can act as strong antioxidants to effectively remove free radicals. They can also regulate carcinogen metabolizing enzymes, inhibit the nitrosation reactions, block the expression of tumor genes, enhance immunity, and therefore act as potent anti-cancer agents[14]. However, a meta-analysis of 12 cohort studies from China, Japan, and America suggested that drinking green tea (≥5 cups per day) was not correlated with the incidence of gastric cancer ($P > 0.05$)[15]. Stratified sex analyses showed that drinking ≥5 cups of green tea per day was a protective factor against gastric cancer in females, but not in males[15]. In this study, results showed that drinking tea lowered the risk of gastric cancer (OR = 0.26, 95% CI: 0.10–0.67), confirming the results shown by Li-Na Mu, et al[16]. Our findings also indicated that drinking tea may be an independent protective factor against gastric cancer, however other factors, such as the type of tea and the water temperature, might affect this relationship and warrant further investigation.

As the medical model shifts from "biomedical" to "bio-psycho-social" paradigm, the relationship between psychological factors and tumor gradually catches the attention of numerous scholars in recent years. A meta-analysis including 5,265 cases of gastric cancer and 12,539 controls from 22 domestic studies found that long-term stress had adverse effects on gastric cancer (merged OR of 2.91, 95% CI: 2.03–4.19)[17]. Another study showed that psychological factors were associated with the incidence of gastric cancer and that history of metal stimulus increased the risk of

gastric cancer significantly (OR = 1.74, 95% CI: 1.11–2.74)[18]. People who often feel anxious/irritable or experience hardships in life have altered hormone levels, which in turn affect their immune system. In particular, epinephrine and norepinephrine are released under stress or anxiety, causing decrease in natural killer cells, reduction in immunity functions and acceleration in the initiation and progression of malignancies[19]. Similar to previous studies, this study also found that often feel troubled was related to gastric cancer (OR = 2.21, 95% CI: 1.23–3.98) in our subjects.

Recent studies indicated that low serum PG I and PG I/II ratio were strongly associated with gastric cancer, while the relationship between PG II and gastric cancer was not as obvious. PG I is primarily secreted by chief cells and mucous neck cells in the fundic glands, whereas PG II is secreted by all gastric glands and the proximal duodenal mucosa[20]. When chronic *H. pylori* infection with chronic atrophic gastritis (CAG) extends from antrum to corpus of stomach, chief cells are replaced by pyloric glands. Therefore, the concentration of serum PG I will decrease due to damaged secretion ability of gastric mucosa, however the secretion of PG II remains, resulting in a lowered PGI/II ratio, which would reflect the severity of CAG. Patients with premalignant lesions, such as CAG or dysplasia, have considerable higher risks for developing gastric cancer. Our previous study[21] explored the changes of serum PG levels in different gastric cancer states. Results found significant differences in serum PG Ⅰ, PG Ⅱ, and PGI/II ratio among the control group, the atrophic gastritis group, and the gastric cancer group (all $P<0.001$). Serum PG Ⅰ level was lower in the gastric cancer group than in the other two groups, and was also lower in the atrophic gastritis group than in the control group, both

differences were statistically significant (P<0.05). Serum PGI/II ratio in the gastric cancer group was lower than in the control group and the atrophic gastritis group, and was also lower in the atrophic gastritis group than in the control group, both differences were statistically significant (P<0.05) . In a study in Japan including 10,996 healthy residents who had underwent gastroscope examinations and tested for serum PG, the incidence of gastric cancer was higher in subjects with lower serum PG I levels and PG I/II ratios[22]. In our study, single conditional logistic regression results showed that serum PG II was higher in gastric cancer patients than in control subjects (P<0.05), PG I/II ratio was lower in gastric cancer patients than in control subjects (P<0.05), and PG I levels in the two groups were not significantly different (P>0.05), which was in agreement with the study conducted by Cao, et al.[23]. Moreover, our results showed that after controlling for other risk factors, decreased levels of serum PG Ⅰ and PG I/II ratios might be independent risk factors for gastric cancer, which was in agreement with the results of a nested case-control study conducted by Kurilovich, et al.[24].

Qin Cao et al[25]. found that serum PG I and PG II levels were lower in patients with advanced-stage gastric cancer than in those with early-stage disease ($P < 0.05$), which was consistent with the results observed in the elderly gastric cancer patients (aged >60 years) by Wei Huang, et al[26]. In this study, serum PG II levels were significantly higher in advanced stage gastric cancer patients than those with early-stage disease ($P < 0.05$), PG I/II ratio was significantly lower in advanced stage gastric cancer patients ($P < 0.05$). These results demonstrated that serum PG Ⅱ level and PG I/II ratio were different at different clinical stages of gastric cancer. Therefore,

the elevation of serum PG II levels and reduced PG I/II ratio may be indicators of the clinical stages of gastric cancer.

Gastrin, a peptide hormone synthesized and secreted by the G cells of the pyloric antrum of the stomach, was first discovered in 1906[27], and is now well known for its multiple subtypes[28]. 90% of the intra-corporeal gastrin is G-17, which is one of the main forms in gastrin circulation, and the change of which can indicate the impairment of gastric mucosa functions[29]. Li Wang et al. [30] had used immunohistochemical method to detect serum G-17, and found it had increased concentrations in superficial gastritis, para-cancerous tissues and gastric cancer cells. In addition, serum G-17 levels were found to be higher in patients with pre-operative gastric cancer than in healthy controls ($P < 0.05$), and were also elevated in patients with advanced clinical stage of gastric cancer[31].

Our finding was coincided with the study conducted by Rui-Xin Lin, el at., that serum G-17 in patients with gastric cancer was higher than in the controls ($P < 0.05$) [32]. This indicated that increased serum G-17 levels were associated with gastric cancer and hypergastrinemia. In terms of the potential mechanism of action, animal experiments with overexpressed G-17 indicated that gastrin could influence gastric carcinogenesis[33]. Serum G-17 levels were found to be higher in patients with gastric corpus cancer than in patients with gastric cancer in other locations ($P < 0.05$), which was similar to the findings described by Hu et al[34]. This may be related to the the vagus nerve's depressive effect on G-17 secretion. Serum G-17 levels were more clearly elevated when cancer invaded the gastric body, which could damage the vagus nerve and inhibit G-17 secretion[35]. Therefore, our results suggested that changes in serum G-17 levels in gastric cancer patients may be indicative of cancer location.

H. pylori infection was a risk factor for gastric cancer. The International Agency for Research on Cancer (IARC) formally identified *H. pylori* as Class I carcinogen in 1994. In addition, a 7.8-year prospective study of 1,526 Japanese patients with gastric diseases showed that patients with *H. pylori* infection had diseases such as gastric atrophy, intestinal metaplasia, as well as precancerous lesions[36]. Moreover, a study conducted in Changle City, Fujian Province, China, showed that although patients with precancerous gastric lesions were unable to avoid gastric cancer, *H. pylori* eradication could reduce the risk of gastric cancer in people without precancerous lesions[37]. The *H. pylori* infection rate in the control group in the current study was 54.4%, which was close to the number found in natural population (54.76%)[38]. However, the infection rate in gastric cancer patients was 66.7%, similar to the results observed by Xue-Yuan Cao, et al[39], suggesting that *H. pylori* infection was more common in gastric cancer patients. In addition, the *H. pylori* infection rate in the gastric cancer group was significantly higher than in the control group in male subjects ($P < 0.05$), whereas no such difference was observed in females, which might be associated with a lower prevalence of *H. pylori* infection in women[40].

There were some strengths in this study. Firstly, Fujian Province is an area with high incidence of gastric cancer in China. According to the report of Fujian Province, the incidence of gastric cancer collected from tumor registration areas in Fujian Province in 2017 was 31.68/100,000, accounting for 12.47% of all new cancer cases, ranking the third in cancer incidence; gastric cancer mortality rate was 25.90/100,000, accounting for 14.74% of all cancer deaths, also ranking the third in cancer deaths. Therefore, Fujian is an important research site for exploring the causes of

gastric cancer. Secondly, strict quality control was conducted in this study in the selection of new cases to make sure the results be comparable. Taking into account the effects of proton pump inhibitors and H receptor antagonists on serum markers, patients who had taken these drugs within a week before recruiting were excluded. With strictly controlling the quality of selected cases, the difficulty in obtaining cases also increased. Thirdly, this study was one of the few studies to use serum indicators as independent variables to explore the risk factors for gastric cancer. However, several limitations should be considered. First, for a case–control study, the causal association between dietary/lifestyle habits and gastric cancer could not be precisely identified. Second, the consumptions of alcohol, tea, and pickled vegetables were self-reported. Subjects often had difficulties in recalling food consumptions and it was also hard to estimate the accurate amount of consumption. Therefore, recall bias and misclassification bias were inevitable. Randomized Control Trial studies shall be conducted for more accurate results. Third, the sample size was relatively small. China has high incidence of gastric cancer and provides favorable conditions for studying it. We plan to expand the sample size in our further researches on gastric cancer.

Conclusion

In conclusion, this study indicated that serum PG and G-17 levels, as well as the detection of *H. pylori* antibodies, could be useful indicators of gastric cancer location and cancer stage. Elevation of serum G-17 levels may be indicative of the gastric cancer location, while the increase in the PG II level and the reduction in the PG I/II ratio may imply the clinical stage. Poor dietary habits, salty food, and often feel troubled may be risk factors for gastric cancer, while eating fresh fruit, onion or garlic, and drinking tea may help protect against this disease.

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Footnotes

Contributions: Lan Lin and Ping Yuan are joint first authors. Kui-cheng Zheng and Wen Wang obtained funding. Kui-cheng Zheng designed the study. Lan Lin drafted the manuscript. Ping Yuan contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content. Wen Wang, Si-han Wu, Liang-xiang Huang collected the data. Bing-shan Wu and Tie-hui Chen detected serological indicators. Xiao-qing Li analyzed the data. All authors have read and approved the final manuscript. Kui-cheng Zheng and Lin Cai are the study guarantors.

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Competing interests: We have read and understood BMJ policy on declaration of interests and declare that we have no competing interests.

Patient consent: Not required.

Ethical approval: This study was approved by the ethical reviews

committee at Fujian Center for Disease Control and Prevention.

Data sharing: Data are stored in Fujian Center for Disease Control and Prevention, No.76 Jintai Road, Gulou District, Fuzhou, China. Data are available upon request to Kui-cheng Zheng; Email address: zkcfjcdc@sina.com.

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Table 1 Single conditional logistic regression analysis on dietary factors, *n(%)*

Factor	Frequency*	Cases	Control	*P*	OR	95% CI
Breakfast consumption	+	112 (62.22)	153 (85.00)		1.00	
	++	38 (21.11)	18 (10.00)	<0.01	2.84	(1.54,5.25)
	+++	30 (16.67)	9 (5.00)	<0.01	4.78	(2.10,10.87)
Meal duration	≥20 min	24 (13.33)	46 (25.56)		1.00	
	10–20 min	50 (27.78)	70 (38.89)	0.393	1.32	(0.70,2.52)
	<20 min	106 (58.89)	64 (35.56)	<0.01	2.97	(1.63,5.40)
Onion or garlic	+	73 (40.56)	49 (27.22)		1.00	
	++	66 (36.67)	56 (31.11)	0.29	0.74	(0.42,1.30)
	+++	41 (22.78)	75 (41.67)	<0.01	0.36	(0.21,0.63)
Spicy food	+	104 (57.78)	125 (69.44)		1.00	
	++	45 (25.00)	48 (26.67)	0.71	1.11	(0.64,1.93)
	+++	31 (17.22)	7 (3.89)	<0.01	5.95	(2.28,15.53)
Hot food	+	76 (42.22)	114 (63.33)		1.00	
	++	47 (26.11)	47 (26.11)	0.25	1.35	(0.81,2.27)
	+++	57 (31.67)	19 (10.56)	<0.01	5.63	(2.72,11.66)
Green vegetables	+	35 (19.44)	15 (8.33)		1.00	
	++	70 (388.9)	46 (25.56)	0.34	0.71	(0.36,1.43)
	+++	75 (41.67)	119 (66.11)	<0.01	0.29	(0.15,0.56)
Fresh fruit	+	115 (63.89)	43 (23.89)		1.00	
	++	42 (23.33)	78 (43.33)	<0.01	0.20	(0.11,0.37)
	+++	23 (12.78)	59 (32.78)	<0.01	0.16	(0.08,0.32)
Dairy	+	133 (73.89)	82 (45.56)		1.00	
	++	32 (17.78)	53 (29.44)	<0.01	0.28	(0.15,0.53)
	+++	15 (8.33)	45 (25.00)	<0.01	0.17	(0.08,0.36)
Pickled vegetables	+	55 (30.56)	125 (69.44)		1.00	
	++	65 (36.11)	45 (25.00)	<0.01	2.67	(1.60,4.49)
	+++	60 (33.33)	10 (5.56)	<0.01	15.27	(5.91,39.49)
Fish sauce	+	109 (60.56)	135 (75.00)		1.00	
	++	30 (16.67)	32 (17.78)	0.96	1.02	(0.56,1.83)
	+++	41 (22.78)	13 (7.22)	<0.01	3.81	(1.89,7.65)

* "+", never or seldom consuming; "++", sometimes consuming; "+++", regularly consuming.

Table 2 Single conditional logistic regression analysis on lifestyle habits and personality factors, *n(%)*

Factors	classified	Cases	Controls	*P*	OR	95% CI
Smoker	No	89 (49.44)	116 (64.44)		1.00	
	Yes	91 (50.56)	64 (35.56)	0.01	2.67	(1.51,4.77)
Alcohol consumption	No	118 (65.56)	140 (77.78)		1.00	
	Yes	62 (34.44)	40 (22.22)	0.01	1.85	(1.15,2.98)
Tea consumption	No	132 (73.33)	102 (56.67)		1.00	
	Yes	48 (26.67)	78 (43.33)	0.01	0.43	(0.23,0.71)
Personality type	Type A	98 (54.44)	78 (43.33)		1.00	
	Intermediate	36 (20.00)	41 (22.78)	0.15	0.66	(0.38,1.15)
	Type B	46 (25.56)	61 (33.89)	0.04	0.59	(0.36,0.97)
Adaptability	Easy	56 (31.11)	61 (33.89)		1.00	
	Between	72 (40.00)	91 (50.56)	0.58	0.88	(0.55,0.41)
	Difficult	52 (28.89)	28 (15.56)	0.03	1.93	(1.08,3.45)
How feel about life	Feel happy	33 (18.33)	63 (35.00)		1.00	
	Feel troubled	71 (39.44)	78 (43.33)	0.03	1.85	(1.06,3.22)
	Often feel troubled	76 (42.22)	39 (21.67)	<0.01	4.16	(2.21,7.86)
Irritable	No	84 (46.67)	110 (61.11)		1.00	
	Yes	96 (53.33)	70 (38.89)	0.04	2.00	(1.25,3.20)
Interpersonal skills	Good	70 (38.89)	96 (53.33)		1.00	
	General	86 (47.78)	75 (41.67)	0.06	1.53	(0.10,2.38)
	Poor	24 (13.33)	9 (5.00)	0.00	3.79	(1.57,9.12)

Table 3 Comparisons of serum PGⅠ, PG Ⅱ, PG I/II ratio and G-17 between cases and controls, M(p25,p75)

Type	Total (n)	PGⅠ (*μg*/L)	PGⅡ (*μg*/L)	PG I/II ratio	G-17 (pmol/L)
Cases	180	115.56 (68.84,177.47)	17.85 (10.58,8.74)	6.58 (4.95,8.48)	7.62 (3.96,14.24)
Controls	180	117.69 (89.83,145.32)	11.19 (7.45,17.37)	9.60 (7.35,13.45)	3.54 (2.24,7.13)
Z	—	**0.26**	**5.48**	**8.06**	**6.01**
P	—	**0.78**	**<0.01**	**<0.01**	**<0.01**

Note: PG is pepsinogen, and G-17 is Gastrin-17

Table 4. Comparison of the H. pylori positive rates between cases and controls, *n(%)*

Type	Cases	Controls	χ^2	*P*
Total	120(66.67)	98(54.44)	5.63	0.02
Gender				
Male	94(52.22)	77(42.78)	4.88	0.03
Female	26(14.44)	21(11.67)	0.97	0.33
χ^2	2.65	1.20		
P	0.10	0.27		
Age, years				
<50	20(11.11)	16(8.89)	0.86	0.36
50–65	51(28.33)	42(23.33)	3.16	0.08
≥65	49(27.22)	40 (22.22)	1.57	0.21
χ^2	**0.55**	**1.28**		
P	**0.76**	**0.53**		

Table 5. Comparison of the serological parameters among patients with different locations of gastric cancer, M(p25,p75)

Location	Cases (n)	PG Ⅰ (*μg*/L)	PG Ⅰ I (*μg*/L)	PG I/II ratio	Gastrin-17 (pmol/L)
Cardia	19	116.15 (61.42,182.66)	17.27 (11.74, 28.83)	6.41 (5.09, 8.01)	6.35 (2.36, 10.75)
Fundus	17	81.26 (53.44, 197.57)	20.72 (9.32, 33.06)	5.22 (3.29, 8.41)	8.14 (3.85, 12.50)
Gastric corpus	55	118.23 (66.41, 175.04)	19.43 (12.25, 28.94)	5.69 (3.78, 8.23)	12.00 (6.58, 19.74)
Gastric antrum	70	118.65 (78.87, 173.66)	16.79 (10.42, 27.49)	7.05 (5.45, 9.07)	6.12 (3.84, 12.67)
Gastric angle	19	116.00 (68.13, 195.76)	15.89 (7.39, 29.28)	7.25 (5.63, 8.10)	6.44 (2.58, 14.81)
χ^2		**1.58**	**1.59**	**7.41**	**10.79**
P		**0.81**	**0.81**	**0.12**	**0.03**

Note: PG is pepsinogen

Table 6. Comparison of the serological parameters in patients with different clinical stages of gastric cancer, M(p25,p75)

Clinical stage	Cases (n)	PG Ⅰ (*μg*/L)	PG ⅠⅠ (*μg*/L)	PG I/II ratio	Gastrin-17 (pmol/L)
Early	49	93.07 (68.39, 171.64)	13.81 (7.42, 23.92)	6.95 (5.71, 9.73)	5.93 (2.85, 12.75)
Advanced	131	118.23 (69.43, 181.24)	19.43 (12.51, 30.41)	6.10 (4.50, 8.03)	8.19 (4.29, 14.39)
Z		**0.88**	**2.33**	**2.43**	**1.75**
P		**0.38**	**0.02**	**0.02**	**0.08**

Note: PG is pepsinogen

Table 7. Multivariate conditional logistic regression analysis.

Factors	β	S.E.	Wald	*P*	OR	95% CI
Hot food	0.84	0.31	7.63	0.006	2.32	(1.28,4.23)
Onion or garlic	-1.05	0.33	10.11	0.001	0.35	(0.18,0.67)
Fresh fruit	-0.60	0.29	4.34	0.037	0.55	(0.31,0.97)
Pickled vegetables	1.39	0.35	15.44	<0.001	4.05	(2.01,8.03)
Tea consumption	-1.35	0.49	7.77	0.005	0.26	(0.10,0.67)
Often feel troubled	0.79	0.30	7.03	0.008	2.21	(1.23,3.98)
PG I	-0.01	0.00	3.96	0.047	0.99	(0.99,1.00)
PG I/II ratio	-0.32	0.07	20.82	<0.001	0.73	(0.63,0.83)

Reporting checklist for genetic association study.

Based on the STREGA guidelines.

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1

Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Background/rationale			
	#2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives			
	#3	State specific objectives, including any prespecified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both.	2
Study design			
	#4	Present key elements of study design early in the paper	2
Setting			
	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2
Eligibility criteria			
	#6a	Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study – Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-	4

		sectional study – Give the eligibility criteria, and the sources and methods of selection of participants. Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	
	#6b	Cohort study – For matched studies, give matching criteria and number of exposed and unexposed. Case-control study – For matched studies, give matching criteria and the number of controls per case.	n/a
Variables			
	#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
	#7b	Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	5
Data sources/measurement			
	#8a	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	4

	#8b	Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory / centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	5
Bias			
	#9a	Describe any efforts to address potential sources of bias	11
	#9b	Describe any efforts to address potential sources of bias	n/a
Study size			
	#10	Explain how the study size was arrived at	4
Quantitative variables			
	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. If applicable, describe how effects of treatment were dealt with.	5
Statistical methods			

#12a	Describe all statistical methods, including those used to control for confounding. State software version used and options (or settings) chosen.	5
#12b	Describe any methods used to examine subgroups and interactions	n/a
#12c	Explain how missing data were addressed	n/a
#12d	If applicable, explain how loss to follow-up was addressed	n/a
#12e	Describe any sensitivity analyses	n/a
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.	n/a
#12g	Describe any methods used for inferring genotypes or haplotypes	n/a
#12h	Describe any methods used to assess or address population stratification.	n/a
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.	n/a
#12j	Describe any methods used to address and correct for relatedness among subjects	n/a

Participants

#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility,	5-6

		confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable. Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	
	#13b	Give reasons for non-participation at each stage	n/a
	#13c	Consider use of a flow diagram	n/a
Descriptive data			
	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. Consider giving information by genotype	5-6
	#14b	Indicate number of participants with missing data for each variable of interest	n/a
	#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.	n/a
Outcome data			
	#15	Cohort study Report numbers of outcome events or summary measures over time.Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each	5-6

		genotype category over time Case-control study – Report numbers in each exposure category, or summary measures of exposure.Give information separately for cases and controls . Report numbers in each genotype category. Cross-sectional study – Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category	
Main results			
	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6
	#16b	Report category boundaries when continuous variables were categorized	n/a
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
	#16d	Report results of any adjustments for multiple comparisons	6
Other analyses			

	#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
	#17b	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
	#17c	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
Key results			
	#18	Summarise key results with reference to study objectives	5-6
Limitations			
	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11
Interpretation			
	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	7-11
Generalisability			
	#21	Discuss the generalisability (external validity) of the study results	7-11
Funding			

#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

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Risk Factors for Gastric Cancer and Related Serological Levels in Fujian, China: Hospital-based Case-control Study

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Risk Factors for Gastric Cancer and Related Serological Levels in Fujian, China: Hospital-based Case-control Study

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Abstract

OBJECTIVE To explore the relationships between gastric cancer and serum PG I, PG II, PG I/II ratio, G-17, and *H. pylori* infection, and to investigate dietary and lifestyle risk factors for gastric cancer in Fujian Province, China.

DESIGN A hospital-based, 1:1 matched case-control study.

SETTING Patients with newly-diagnosed gastric cancer were recruited from the Fujian Provincial Hospital and the No.900 Hospital of the Joint Support Force of the Chinese People's Liberation Army between July 2014 and December 2016.

PARTICIPANTS A total of 180 pairs of gastric cancer patients and control subjects were recruited in the study, including 134 (74.4%) male pairs and 46 (25.6%) female pairs.

INVESTIGATION AND ANALYSIS MEASURES Serological indicators were tested with ELISA kits. Dietary, lifestyle and psychological factors were investigated through face-to-face questionnaire. Relationships between gastric cancer and these influencing factors were examined by Chi-square test and conditional logistic regression.

RESULTS Serum PG II and G-17 levels and *H. pylori* infection rate were higher in gastric cancer patients than in control subjects ($P < 0.05$), while PG I/II ratio was lower in gastric cancer patients ($P < 0.05$). Serum G-17 levels were higher in patients with corpus gastric cancer than in patients with antral gastric cancer ($P < 0.05$). Serum PG II levels were higher in patients with advanced gastric cancer than in patients with early-stage cancer ($P < 0.05$), however, PG I/II ratio was lower in patients with advanced stage gastric cancer than in patients with early-stage cancer ($P < 0.05$). Eating hot food (OR= 2.32), eating pickled vegetables (OR = 4.05), and often feel troubled (OR = 2.21) were found to significantly increase the risk of gastric cancer (all $P < 0.05$), while

consuming onion or garlic (OR = 0.35), drinking tea (OR = 0.26), eating fresh fruit (OR = 0.55), and high serum PG I (OR = 0.99) or PG I/II ratio (OR = 0.73) were found to be protective against gastric cancer.

CONCLUSION Study results showed that serum PG, G-17 and *H. pylori* antibodies could be useful indicators for early diagnosis of gastric cancer. Increase in serum G-17 level might indicate the location of gastric cancer. Increase in serum PG II level and decrease in PG I/II ratio might imply the clinical stage. Eating hot food, eating pickled vegetables, and often feel troubled may be risk factors for gastric cancer, while eating fresh fruit, eating onion or garlic, and drinking tea may be protective factors against the disease.

Key words: Gastric cancer, Risk factor, Pepsinogen, Gastrin, *Helicobacter pylori*

Strengths and limitations of study

Fujian Province, high in gastric cancer incidences, is an important research site for exploring the etiologies of gastric cancer.

This study was one of the few studies to use serum indicators as independent variables to analyze the risk factors for gastric cancer.

Strict quality control was conducted in the selection of new cases to ensure comparable results.

However, as a case–control study, recall bias was inevitable and trial studies are required for more accurate results.

Sample size of this study was not large enough, further studies will recruit more subjects.

INTRODUCTION

Gastric cancer is a common malignancy in the gastric mucosa and gastric glands in the digestive tract and accounts for a high proportion of cancer deaths in China, especially in Fujian Province. According to the World Cancer Report published by the World Health Organization in 2018[1], there were 1,033,071 new cases of gastric cancer (accounting for 5.7% of all cancer incidence) and 782,685 deaths from it (accounting for 8.2% of all cancer deaths) worldwide. Gastric cancer has high disease burdens[2], ranking second only to lung cancer in terms of the number of deaths[1]. In China, there were 456,124 new cases of gastric cancer in 2018, accounting for 44.1% of global incidence[3]. In Fujian Province, located in southeast China, gastric cancer accounted for 12.5% of all cancer incidences in 2014[4].Risk factors for gastric cancer such as dietary, lifestyle, and psychological factors are different across China[5-10]. The overall objectives of this study were to explore the relationship between gastric cancer and serum PG Ⅰ, PG Ⅱ, PG Ⅰ/Ⅱ ratio, G-17 and *H.pylori* infection, and to investigate the risk factors for gastric cancer in Fujian.

METHODS

Study design

This was a hospital-based, 1:1 matched case-control study, performed in accordance to the Declaration of Helsinki of the World Medical Association and approved by the Ethics Committee of the Fujian Center for Disease Control and Prevention. Informed consents were obtained from all recruited subjects. Subjects' dietary and lifestyle data were obtained through face-to-face interviews by trained investigators, and blood samples were collected for the test of serum markers.

Case and control groups

From July 2014 to December 2016, patients with newly-diagnosed gastric cancer were recruited from the Fujian Provincial Hospital and the No.900 Hospital of the Joint Support Force of the Chinese People's Liberation Army. Control subjects were recruited simultaneously from the Medical Examination Center at the same hospital. Gastric patients and control subjects were 1:1 matched according to gender and similar age within 3 years. Inclusion criteria for the gastric cancer group were: newly diagnosed of gastric cancer by gastroscopy and pathology, with no medication history of antibiotics, proton pump inhibitors, or H receptor antagonists. Patients with prior surgeries or chemotherapies were also excluded. Inclusion criteria for the control group were: no diagnosis or symptoms of chronic stomach diseases, and no mental retardation or emotional blockages. All subjects had lived in Fujian Province for more than 10 years.

Risk Factor Survey

Data of risk factors were collected by trained investigators through face-to-face interviews with the subjects, using a consolidated questionnaire. The survey contents included general information, dietary habits(i.e., breakfast consumption, meal duration, intake frequencies of onion or garlic, spicy food, hot food, green vegetables, fresh fruit, dairy, pickled vegetables, and fish sauce), lifestyle habits (i.e., smoking, alcohol consumption, and tea consumption), and psychological factors (i.e., personality type, adaptability, feels about life, and interpersonal skills).

Serum sampling

A total of 5 mL of fasting venous blood was collected from each subject, and was centrifuged at 3000 r/min for 10 min to extract the serum, which was then stored at -80℃ for further testing. Serum PG I, PG II, and G-17 levels were tested using ELISA kits from BIOHIT, Finland (batch numbers

of 19PA1505, 21PB1508, and 19GC1506, respectively). Antibodies to *H. pylori* were assessed using ELISA kits from the AI Kang Company of China (lot number 201510087).

Definitions and variables

Dietary intake frequencies were classified as: never or seldom consumption (no more than once per week), occasional consumption (two to six times per week), and regular consumption (seven or more times per week). Smokers were defined as subjects who had smoked more than 100 cigarettes overall. Alcohol consumers were defined as subjects who had been drinking any alcohol at least once a week for more than six months. Tea consumers were defined as subjects who had been drinking any tea at least once a week for more than six months.

According to the calculated cut-off value, an optical density (OD) value of the sample >1.1 × the cut-off value was considered *H. pylori*-positive, while an OD <0.9 × the cut-off value was considered *H. pylori*-negative.

Statistical analysis

Data were inputted by the double-entry method and tested for consistency using Epidata 3.1. The SPSS version 24.0 software package was used for conducting Chi-squared tests on the demographic information, and for conducting the single and multivariate conditional logistic regression analyses on the other information to determine the odds ratio (OR) and 95% confidence interval (CI). Test significance level was set at 0.05.

Patient and public involvement

Dr Kazuo Aoki, a Japanese expert, was involved in designing the study due to the growing concern among people with Gastric Cancer both in

Japan and China. This study was undertaken in the two hospitals with the First-class at Grade 3 in Fujian province in order to ensure the quality of research and access to adequate and widely sourced cases. The findings of this study will be published to inform the public about the risk factors for gastric cancer and some of the inspection results were fed back to the subjects through the form of inspection report.

RESULTS

Demographic information

A total of 180 pairs of gastric cancer patients and control subjects were recruited in this study, of which 134 pairs (74.4%) were male subjects and 46 pairs (25.6%) were female subjects. The average ages of the cancer group and the control group were 61.0 ± 10.8 and 60.1 ± 10.9 years, respectively. The two groups were not significantly different in age, marital status, education, or labor intensity (all $P \geq 0.05$), however the occupation compositions were different.

Dietary habits, lifestyles and personality traits

As shown in Table 1, there were significant differences in the dietary habits between cases and controls. Lifestyles, such as smoking, alcohol consumption, and tea consumption, of the two groups were also significantly different (Table 2).

Personality types, adaptability, feelings about life, irritability, and interpersonal skills were also significantly different between cases and controls (Table 2).

Serum PG I, PG II, PG I/II ratio and G-17 levels

Serum PG I, PG II, and G-17 levels did not fit normal distributions, nor did

the PG I/II ratio; therefore median values were used to indicate central tendencies and the P25 and P75 percentiles were used to indicate dispersion tendencies. Serum PG II and G-17 levels in gastric cancer patients were higher than those in the control subjects ($P < 0.05$), while the PG I/II ratio was lower in the patients ($P < 0.05$); PG I levels were not statistically different between the two groups. The results of the serum markers are summarized in Table 3.

H. pylori infection

As shown in Table 4, 66.67% of gastric cancer patients were *H. pylori*-positive, which was significantly higher than that of the control group (54.44%, $P < 0.05$). The positive rate of *H. pylori* was higher in male gastric cancer patients than in controls ($P < 0.05$), however no significant differences were found between female cases and controls or among age groups ($P > 0.05$).

Serological parameters with different locations of gastric cancer

Serum G-17 levels were higher in patients with gastric corpus cancer than in patients with gastric antrum cancer ($P < 0.05$), but not significantly different among patients with other tumor locations ($P > 0.05$; Table 5). Serum PG I, PG II levels, and the PG I/II ratio were not statistically different among patients with different tumor locations ($P > 0.05$; Table 5).

Serological parameters with different clinical stages of gastric cancer

As shown in Table 6, serum PG II levels in patients with advanced gastric cancer were higher than in patients with early-stage gastric cancer ($P < 0.05$). However, the PG I/II ratio was lower in patients with advanced cancer than in patients with early-stage disease ($P < 0.05$). Serum PG I and

G-17 levels were not significantly different between the two different clinical stages ($P > 0.05$).

Multivariate analysis

Multivariate conditional logistic regression analysis was performed with gastric cancer as the independent variable, and the dietary/lifestyle habits, psychological factors, serum PG I level, serum PG II level, PG I/II ratio, serum G-17 level, and *H. pylori* infection as dependent variables. As shown in Table 7, eating hot food, consumption of pickled vegetables, and often feel troubled may be risk factors for gastric cancer. However, the consumption of fresh fruit, onion or garlic, drinking tea, and elevated serum PG I levels and PG I/II ratio might be protective factors for gastric cancer. Other factors examined in this study had no statistically significant effect on gastric cancer ($P > 0.05$).

DISCUSSION

Overall, this study has found that gastric cancer was related to several dietary and lifestyle factors, the changes in serum PG I/II ratio and G-17, and the infection rate of *H. pylori*.

Multivariate conditional logistic regression analysis indicated that the consumption of pickled vegetables may be a risk factor for gastric cancer. Pickles are high in salt and may damage the gastric mucosa, reduce gastric acid secretion, and inhibit the synthesis of prostaglandin E, which enhances gastric mucosa resistance[11]. In addition, pickles contain high amount of nitrate and nitrite, which can be converted to N-nitrosamide, a

carcinogen, under gastric acid conditions[12]. In an area with high incidence of gastric cancer in northwest China's Gansu Province[13], where people often consume pickled vegetables as substitutions of fresh vegetables in winters, 65%-75% of pickles had detectable levels of nitrite and all had tested positive for nitrate; and the levels of nitrite and nitrate in the gastric juice were significantly higher in people often consume pickled vegetables than in people who seldom consume pickled vegetables. Nitrate and nitrite levels in the gastric juice were associated with the frequency of pickle intake, which in turn was associated with gastric cancer. This study also found that regular pickle intake (seven or more times per week) increased the risk of gastric cancer (OR = 4.05; 95% CI: 2.01–8.02).

Study results also indicated that tea consumption was a protective factor against gastric cancer. Tie Guanyin, one of the most popular teas in Fujian, is rich in tea polyphenols. Tea polyphenols can act as strong antioxidants to effectively remove free radicals. They can also regulate carcinogen metabolizing enzymes, inhibit the nitrosation reactions, block the expression of tumor genes, enhance immunity, and therefore act as potent anti-cancer agents[14]. However, a meta-analysis of 12 cohort studies from China, Japan, and America suggested that drinking green tea (≥5 cups per day) was not correlated with the incidence of gastric cancer ($P > 0.05$)[15]. Stratified sex analyses showed that drinking ≥5 cups of green tea per day was a protective factor against gastric cancer in females, but not in males[15]. In this study, results showed that drinking tea lowered the risk of gastric cancer (OR = 0.26, 95% CI: 0.10–0.67), confirming the results shown by Li-Na Mu, et al[16]. Our findings also indicated that drinking tea may be an independent protective factor against gastric cancer, however other factors, such as the type of tea and the water temperature, might affect this relationship and warrant further investigation.

As the medical model shifts from "biomedical" to "bio-psycho-social" paradigm, the relationship between psychological factors and tumor gradually catches the attention of numerous scholars in recent years. A meta-analysis including 5,265 cases of gastric cancer and 12,539 controls from 22 domestic studies found that long-term stress had adverse effects on gastric cancer (merged OR of 2.91, 95% CI: 2.03–4.19)[17]. Another study showed that psychological factors were associated with the incidence of gastric cancer and that history of metal stimulus increased the risk of gastric cancer significantly (OR = 1.74, 95% CI: 1.11–2.74)[18]. People who often feel anxious/irritable or experience hardships in life have altered hormone levels, which in turn affect their immune system. In particular, epinephrine and norepinephrine are released under stress or anxiety, causing decrease in natural killer cells, reduction in immunity functions and acceleration in the initiation and progression of malignancies[19]. Similar to previous studies, this study also found that often feel troubled was related to gastric cancer (OR = 2.21, 95% CI: 1.23–3.98) in our subjects.

Recent studies indicated that low serum PG I and PG I/II ratio were strongly associated with gastric cancer, while the relationship between PG II and gastric cancer was not as obvious. PG I is primarily secreted by chief cells and mucous neck cells in the fundic glands, whereas PG II is secreted by all gastric glands and the proximal duodenal mucosa[20]. When chronic *H. pylori* infection with chronic atrophic gastritis (CAG) extends from antrum to corpus of stomach, chief cells are replaced by pyloric glands. Therefore, the concentration of serum PG I will decrease due to damaged secretion ability of gastric mucosa, however the secretion of PG II remains, resulting in a lowered PGI/II ratio, which would reflect the

severity of CAG. Patients with premalignant lesions, such as CAG or dysplasia, have considerable higher risks for developing gastric cancer. Our previous study[21] explored the changes of serum PG levels in different gastric cancer states. Results found significant differences in serum PG Ⅰ, PG Ⅱ, and PGI/II ratio among the control group, the atrophic gastritis group, and the gastric cancer group (all P<0.001). Serum PG Ⅰ level was lower in the gastric cancer group than in the other two groups, and was also lower in the atrophic gastritis group than in the control group, both differences were statistically significant (P<0.05). Serum PGI/II ratio in the gastric cancer group was lower than in the control group and the atrophic gastritis group, and was also lower in the atrophic gastritis group than in the control group, both differences were statistically significant (P<0.05) . In a study in Japan including 10,996 healthy residents who had underwent gastroscope examinations and tested for serum PG, the incidence of gastric cancer was higher in subjects with lower serum PG I levels and PG I/II ratios[22]. In our study, single conditional logistic regression results showed that serum PG II was higher in gastric cancer patients than in control subjects (P<0.05), PG I/II ratio was lower in gastric cancer patients than in control subjects (P<0.05), and PG I levels in the two groups were not significantly different (P>0.05), which was in agreement with the study conducted by Cao, et al.[23]. Moreover, our results showed that after controlling for other risk factors, decreased levels of serum PG Ⅰ and PG I/II ratios might be independent risk factors for gastric cancer, which was in agreement with the results of a nested case-control study conducted by Kurilovich, et al.[24].

Qin Cao et al[25]. found that serum PG I and PG II levels were lower in patients with advanced-stage gastric cancer than in those with early-stage

disease ($P < 0.05$), which was consistent with the results observed in the elderly gastric cancer patients (aged >60 years) by Wei Huang, et al[26]. In this study, serum PG II levels were significantly higher in advanced stage gastric cancer patients than those with early-stage disease ($P < 0.05$), PG I/II ratio was significantly lower in advanced stage gastric cancer patients ($P < 0.05$). These results demonstrated that serum PG Ⅱ level and PG I/II ratio were different at different clinical stages of gastric cancer. Therefore, the elevation of serum PG II levels and reduced PG I/II ratio may be indicators of the clinical stages of gastric cancer.

Gastrin, a peptide hormone synthesized and secreted by the G cells of the pyloric antrum of the stomach, was first discovered in 1906[27], and is now well known for its multiple subtypes[28]. 90% of the intra-corporeal gastrin is G-17, which is one of the main forms in gastrin circulation, and the change of which can indicate the impairment of gastric mucosa functions[29]. Li Wang et al. [30] had used immunohistochemical method to detect serum G-17, and found it had increased concentrations in superficial gastritis, para-cancerous tissues and gastric cancer cells. In addition, serum G-17 levels were found to be higher in patients with pre-operative gastric cancer than in healthy controls ($P < 0.05$), and were also elevated in patients with advanced clinical stage of gastric cancer[31].

Our finding was coincided with the study conducted by Rui-Xin Lin, el at., that serum G-17 in patients with gastric cancer was higher than in the controls ($P < 0.05$) [32]. This indicated that increased serum G-17 levels were associated with gastric cancer and hypergastrinemia. In terms of the potential mechanism of action, animal experiments with overexpressed G-17 indicated that gastrin could influence gastric carcinogenesis[33]. Serum G-17 levels were found to be higher in patients with gastric corpus

cancer than in patients with gastric cancer in other locations ($P < 0.05$), which was similar to the findings described by Hu et al[34]. This may be related to the the vagus nerve's depressive effect on G-17 secretion. Serum G-17 levels were more clearly elevated when cancer invaded the gastric body, which could damage the vagus nerve and inhibit G-17 secretion[35]. Therefore, our results suggested that changes in serum G-17 levels in gastric cancer patients may be indicative of cancer location.

H. pylori infection was a risk factor for gastric cancer. The International Agency for Research on Cancer (IARC) formally identified *H. pylori* as Class I carcinogen in 1994. In addition, a 7.8-year prospective study of 1,526 Japanese patients with gastric diseases showed that patients with *H. pylori* infection had diseases such as gastric atrophy, intestinal metaplasia, as well as precancerous lesions[36]. Moreover, a study conducted in Changle City, Fujian Province, China, showed that although patients with precancerous gastric lesions were unable to avoid gastric cancer, *H. pylori* eradication could reduce the risk of gastric cancer in people without precancerous lesions[37]. The *H. pylori* infection rate in the control group in the current study was 54.4%, which was close to the number found in natural population (54.76%)[38]. However, the infection rate in gastric cancer patients was 66.7%, similar to the results observed by Xue-Yuan Cao, et al[39], suggesting that *H. pylori* infection was more common in gastric cancer patients. In addition, the *H. pylori* infection rate in the gastric cancer group was significantly higher than in the control group in male subjects ($P < 0.05$), whereas no such difference was observed in females, which might be associated with a lower prevalence of *H. pylori* infection in women[40].

There were some strengths in this study. Firstly, Fujian Province is an area

with high incidence of gastric cancer in China. According to the report of Fujian Province, the incidence of gastric cancer collected from tumor registration areas in Fujian Province in 2017 was 31.68/100,000, accounting for 12.47% of all new cancer cases, ranking the third in cancer incidence; gastric cancer mortality rate was 25.90/100,000, accounting for 14.74% of all cancer deaths, also ranking the third in cancer deaths. Therefore, Fujian is an important research site for exploring the causes of gastric cancer. Secondly, strict quality control was conducted in this study in the selection of new cases to make sure the results be comparable. Taking into account the effects of proton pump inhibitors and H receptor antagonists on serum markers, patients who had taken these drugs within a week before recruiting were excluded. With strictly controlling the quality of selected cases, the difficulty in obtaining cases also increased. Thirdly, this study was one of the few studies to use serum indicators as independent variables to explore the risk factors for gastric cancer. However, several limitations should be considered. First, for a case–control study, the causal association between dietary/lifestyle habits and gastric cancer could not be precisely identified. Second, the consumptions of alcohol, tea, and pickled vegetables were self-reported. Subjects often had difficulties in recalling food consumptions and it was also hard to estimate the accurate amount of consumption. Therefore, recall bias and misclassification bias were inevitable. Randomized Control Trial studies shall be conducted for more accurate results. Third, the sample size was relatively small. China has high incidence of gastric cancer and provides favorable conditions for studying it. We plan to expand the sample size in our further researches on gastric cancer.

Conclusion

In conclusion, this study indicated that serum PG and G-17 levels, as well

as the detection of *H. pylori* antibodies, could be useful indicators of gastric cancer location and cancer stage. Elevation of serum G-17 levels may be indicative of the gastric cancer location, while the increase in the PG II level and the reduction in the PG I/II ratio may imply the clinical stage. Poor dietary habits, salty food, and often feel troubled may be risk factors for gastric cancer, while eating fresh fruit, onion or garlic, and drinking tea may help protect against this disease.

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Footnotes

Contributions: PY and LL are joint first authors. KZ and WW obtained funding. KZ designed the study. LL drafted the manuscript. PY contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content. WW, SW, LH collected the data. BW and T C detected serological indicators. XL analyzed the data. All authors have read and approved the final manuscript. KZ and LC are the study guarantors.

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Competing interests: We have read and understood BMJ policy on declaration of interests and declare that we have no competing interests.

Patient consent: All subjects signed the informed consent form.

Ethical approval: This study was approved by the ethical reviews committee at Fujian Center for Disease Control and Prevention.

Data sharing: Data are stored in Fujian Center for Disease Control and Prevention, No.76 Jintai Road, Gulou District, Fuzhou, China. Data are available upon request to Kui-cheng Zheng; Email address: zkcfjcdc@sina.com.

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Table 1 Single conditional logistic regression analysis on dietary factors, *n(%)*

Factor	Frequency*	Cases	Control	*P*	OR	95% CI
Breakfast consumption	+	112 (62.22)	153 (85.00)		1.00	
	++	38 (21.11)	18 (10.00)	<0.01	2.84	(1.54,5.25)
	+++	30 (16.67)	9 (5.00)	<0.01	4.78	(2.10,10.87)
Meal duration	≥20 min	24 (13.33)	46 (25.56)		1.00	
	10–20 min	50 (27.78)	70 (38.89)	0.393	1.32	(0.70,2.52)
	<20 min	106 (58.89)	64 (35.56)	<0.01	2.97	(1.63,5.40)
Onion or garlic	+	73 (40.56)	49 (27.22)		1.00	
	++	66 (36.67)	56 (31.11)	0.29	0.74	(0.42,1.30)
	+++	41 (22.78)	75 (41.67)	<0.01	0.36	(0.21,0.63)
Spicy food	+	104 (57.78)	125 (69.44)		1.00	
	++	45 (25.00)	48 (26.67)	0.71	1.11	(0.64,1.93)
	+++	31 (17.22)	7 (3.89)	<0.01	5.95	(2.28,15.53)
Hot food	+	76 (42.22)	114 (63.33)		1.00	
	++	47 (26.11)	47 (26.11)	0.25	1.35	(0.81,2.27)
	+++	57 (31.67)	19 (10.56)	<0.01	5.63	(2.72,11.66)
Green vegetables	+	35 (19.44)	15 (8.33)		1.00	
	++	70 (388.9)	46 (25.56)	0.34	0.71	(0.36,1.43)
	+++	75 (41.67)	119 (66.11)	<0.01	0.29	(0.15,0.56)
Fresh fruit	+	115 (63.89)	43 (23.89)		1.00	
	++	42 (23.33)	78 (43.33)	<0.01	0.20	(0.11,0.37)
	+++	23 (12.78)	59 (32.78)	<0.01	0.16	(0.08,0.32)
Dairy	+	133 (73.89)	82 (45.56)		1.00	
	++	32 (17.78)	53 (29.44)	<0.01	0.28	(0.15,0.53)
	+++	15 (8.33)	45 (25.00)	<0.01	0.17	(0.08,0.36)
Pickled vegetables	+	55 (30.56)	125 (69.44)		1.00	
	++	65 (36.11)	45 (25.00)	<0.01	2.67	(1.60,4.49)
	+++	60 (33.33)	10 (5.56)	<0.01	15.27	(5.91,39.49)
Fish sauce	+	109 (60.56)	135 (75.00)		1.00	
	++	30 (16.67)	32 (17.78)	0.96	1.02	(0.56,1.83)
	+++	41 (22.78)	13 (7.22)	<0.01	3.81	(1.89,7.65)

* "+", never or seldom consuming; "++", sometimes consuming; "+++", regularly consuming.

Table 2 Single conditional logistic regression analysis on lifestyle habits and personality factors, *n(%)*

Factors	classified	Cases	Controls	*P*	OR	95% CI
Smoker	No	89 (49.44)	116 (64.44)		1.00	
	Yes	91 (50.56)	64 (35.56)	0.01	2.67	(1.51,4.77)
Alcohol consumption	No	118 (65.56)	140 (77.78)		1.00	
	Yes	62 (34.44)	40 (22.22)	0.01	1.85	(1.15,2.98)
Tea consumption	No	132 (73.33)	102 (56.67)		1.00	
	Yes	48 (26.67)	78 (43.33)	0.01	0.43	(0.23,0.71)
Personality type	Type A	98 (54.44)	78 (43.33)		1.00	
	Intermediate	36 (20.00)	41 (22.78)	0.15	0.66	(0.38,1.15)
	Type B	46 (25.56)	61 (33.89)	0.04	0.59	(0.36,0.97)
Adaptability	Easy	56 (31.11)	61 (33.89)		1.00	
	Between	72 (40.00)	91 (50.56)	0.58	0.88	(0.55,0.41)
	Difficult	52 (28.89)	28 (15.56)	0.03	1.93	(1.08,3.45)
How feel about life	Feel happy	33 (18.33)	63 (35.00)		1.00	
	Feel troubled	71 (39.44)	78 (43.33)	0.03	1.85	(1.06,3.22)
	Often feel troubled	76 (42.22)	39 (21.67)	<0.01	4.16	(2.21,7.86)
Irritable	No	84 (46.67)	110 (61.11)		1.00	
	Yes	96 (53.33)	70 (38.89)	0.04	2.00	(1.25,3.20)
Interpersonal skills	Good	70 (38.89)	96 (53.33)		1.00	
	General	86 (47.78)	75 (41.67)	0.06	1.53	(0.10,2.38)
	Poor	24 (13.33)	9 (5.00)	0.00	3.79	(1.57,9.12)

Table 3 Comparisons of serum PGⅠ, PGⅡ, PG I/II ratio and G-17 between cases and controls, M(p25,p75)

Type	Total (n)	PGⅠ (*μg*/L)	PGⅡ (*μg*/L)	PG I/II ratio	G-17 (pmol/L)
Cases	180	115.56 (68.84,177.47)	17.85 (10.58,8.74)	6.58 (4.95,8.48)	7.62 (3.96,14.24)
Controls	180	117.69 (89.83,145.32)	11.19 (7.45,17.37)	9.60 (7.35,13.45)	3.54 (2.24,7.13)
Z	—	**0.26**	**5.48**	**8.06**	**6.01**
P	—	**0.78**	**<0.01**	**<0.01**	**<0.01**

Note: PG is pepsinogen, and G-17 is Gastrin-17

Table 4. Comparison of the H. pylori positive rates between cases and controls, *n(%)*

Type	Cases	Controls	χ^2	*P*
Total	120(66.67)	98(54.44)	5.63	0.02
Gender				
Male	94(52.22)	77(42.78)	4.88	0.03
Female	26(14.44)	21(11.67)	0.97	0.33
χ^2	2.65	1.20		
P	0.10	0.27		
Age, years				
<50	20(11.11)	16(8.89)	0.86	0.36
50–65	51(28.33)	42(23.33)	3.16	0.08
≥65	49(27.22)	40 (22.22)	1.57	0.21
χ^2	**0.55**	**1.28**		
P	**0.76**	**0.53**		

Table 5. Comparison of the serological parameters among patients with different locations of gastric cancer, M(p25,p75)

Location	Cases (n)	PG Ⅰ (*μg*/L)	PG Ⅰ I (*μg*/L)	PG I/II ratio	Gastrin-17 (pmol/L)
Cardia	19	116.15 (61.42,182.66)	17.27 (11.74, 28.83)	6.41 (5.09, 8.01)	6.35 (2.36, 10.75)
Fundus	17	81.26 (53.44, 197.57)	20.72 (9.32, 33.06)	5.22 (3.29, 8.41)	8.14 (3.85, 12.50)
Gastric corpus	55	118.23 (66.41, 175.04)	19.43 (12.25, 28.94)	5.69 (3.78, 8.23)	12.00 (6.58, 19.74)
Gastric antrum	70	118.65 (78.87, 173.66)	16.79 (10.42, 27.49)	7.05 (5.45, 9.07)	6.12 (3.84, 12.67)
Gastric angle	19	116.00 (68.13, 195.76)	15.89 (7.39, 29.28)	7.25 (5.63, 8.10)	6.44 (2.58, 14.81)
χ^2		**1.58**	**1.59**	**7.41**	**10.79**
P		**0.81**	**0.81**	**0.12**	**0.03**

Note: PG is pepsinogen

Table 6. Comparison of the serological parameters in patients with different clinical stages of gastric cancer, M(p25,p75)

Clinical stage	Cases (n)	PG Ⅰ (*μg*/L)	PG ⅠⅠ (*μg*/L)	PG I/II ratio	Gastrin-17 (pmol/L)
Early	49	93.07 (68.39, 171.64)	13.81 (7.42, 23.92)	6.95 (5.71, 9.73)	5.93 (2.85, 12.75)
Advanced	131	118.23 (69.43, 181.24)	19.43 (12.51, 30.41)	6.10 (4.50, 8.03)	8.19 (4.29, 14.39)
Z		**0.88**	**2.33**	**2.43**	**1.75**
P		**0.38**	**0.02**	**0.02**	**0.08**

Note: PG is pepsinogen

Table 7. Multivariate conditional logistic regression analysis.

Factors	β	S.E.	Wald	*P*	OR	95% CI
Hot food	0.84	0.31	7.63	0.006	2.32	(1.28,4.23)
Onion or garlic	-1.05	0.33	10.11	0.001	0.35	(0.18,0.67)
Fresh fruit	-0.60	0.29	4.34	0.037	0.55	(0.31,0.97)
Pickled vegetables	1.39	0.35	15.44	<0.001	4.05	(2.01,8.03)
Tea consumption	-1.35	0.49	7.77	0.005	0.26	(0.10,0.67)
Often feel troubled	0.79	0.30	7.03	0.008	2.21	(1.23,3.98)
PG I	-0.01	0.00	3.96	0.047	0.99	(0.99,1.00)
PG I/II ratio	-0.32	0.07	20.82	<0.001	0.73	(0.63,0.83)

Reporting checklist for genetic association study.

Based on the STREGA guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1

Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Background/rationale			
	#2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives			
	#3	State specific objectives, including any prespecified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both.	2
Study design			
	#4	Present key elements of study design early in the paper	2
Setting			
	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2
Eligibility criteria			
	#6a	Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study – Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-	4

		sectional study – Give the eligibility criteria, and the sources and methods of selection of participants. Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	
	#6b	Cohort study – For matched studies, give matching criteria and number of exposed and unexposed. Case-control study – For matched studies, give matching criteria and the number of controls per case.	n/a
Variables			
	#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
	#7b	Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	5
Data sources/measurement			
	#8a	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	4

	#8b	Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory / centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	5
Bias			
	#9a	Describe any efforts to address potential sources of bias	11
	#9b	Describe any efforts to address potential sources of bias	n/a
Study size			
	#10	Explain how the study size was arrived at	4
Quantitative variables			
	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. If applicable, describe how effects of treatment were dealt with.	5
Statistical methods			

#12a	Describe all statistical methods, including those used to control for confounding. State software version used and options (or settings) chosen.	5
#12b	Describe any methods used to examine subgroups and interactions	n/a
#12c	Explain how missing data were addressed	n/a
#12d	If applicable, explain how loss to follow-up was addressed	n/a
#12e	Describe any sensitivity analyses	n/a
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.	n/a
#12g	Describe any methods used for inferring genotypes or haplotypes	n/a
#12h	Describe any methods used to assess or address population stratification.	n/a
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.	n/a
#12j	Describe any methods used to address and correct for relatedness among subjects	n/a

Participants

#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility,	5-6

		confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable. Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	
	#13b	Give reasons for non-participation at each stage	n/a
	#13c	Consider use of a flow diagram	n/a
Descriptive data			
	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. Consider giving information by genotype	5-6
	#14b	Indicate number of participants with missing data for each variable of interest	n/a
	#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.	n/a
Outcome data			
	#15	Cohort study Report numbers of outcome events or summary measures over time.Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each	5-6

		genotype category over time Case-control study – Report numbers in each exposure category, or summary measures of exposure.Give information separately for cases and controls . Report numbers in each genotype category. Cross-sectional study – Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category	
Main results			
	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6
	#16b	Report category boundaries when continuous variables were categorized	n/a
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
	#16d	Report results of any adjustments for multiple comparisons	6
Other analyses			

	#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
	#17b	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
	#17c	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
Key results			
	#18	Summarise key results with reference to study objectives	5-6
Limitations			
	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11
Interpretation			
	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	7-11
Generalisability			
	#21	Discuss the generalisability (external validity) of the study results	7-11
Funding			

#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11