ORIGINAL ARTICLE

Journal of Digestive Diseases   WILEY

Bile reflux is an independent risk factor for precancerous gastric lesions and gastric cancer: An observational cross-sectional study

Lu Yao Zhang[1],* 🔘 | Jian Zhang[1,2],* | Dan Li[3],* 🔘 | Yuan Liu[4],* | Dong Ling Zhang[3] | Cai Fang Liu[5] | Na Wang[1] | Si Ran Wu[1] | Wen Quan Lu[6] | Jing Zhi Guo[7] | Yong Quan Shi[1] 🔘

[1]State Key Laboratory of Cancer Biology, National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Air Force Medical University, Xi'an, Shaanxi Province, China

[2]Department of Gastroenterology, Air Force Hospital of Northern Theater of PLA, Shenyang, Liaoning Province, China

[3]Department of General Practice, First Affiliated Hospital of Xi'an Medical University, Xi'an, Shaanxi Province, China

[4]Department of Emergency Medicine, Shaanxi Xin'an Central Hospital, Xi'an, Shaanxi Province, China

[5]Department of Pediatrics, First Affiliated Hospital of Xi'an Medical University, Shaanxi Province, China

[6]Department of Gastroenterology, Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China

[7]Department of Anesthesiology, Xijing Hospital, Air Force Medical University, Xi'an, Shaanxi Province, China

Correspondence
Yong Quan Shi, State Key Laboratory of Cancer Biology, National Clinical Research Center for Digestive Diseases, Xijing Hospital of Digestive Diseases, Air Force Medical University, No. 127 West Changle Road, Xi'an, Shaanxi Province 710032, China.
Email: shiyquan@fmmu.edu.cn

Objective: To identify whether bile reflux on endoscopy and other related variables are risk factors for precancerous gastric lesions and gastric cancer (GC).

Methods: A multicenter, cross-sectional and observational study was conducted in five centers in China from June to October 2019, 1162 patients were recruited and divided into the chronic gastritis (CG), the precancerous lesion (low-grade intra-epithelial neoplasia and intestinal metaplasia), and GC groups (including high-grade intraepithelial neoplasia). All participants underwent detailed interviews, endoscopy and biopsy, and completed questionnaires. Odds ratio and 95% confidence interval were calculated with multivariate logistic regression models with or without adjustment for *Helicobacter pylori* infection.

Results: We recruited 668 patients with CG, 411 with precancerous lesions and 83 with GC. By comparing the CG and precancerous lesion groups, independent risk factors for cancerous gastric lesions were the grade of bile reflux, patient's age, dietary habits and family history of GC. Similar results were obtained when comparing the CG and GC groups. In addition, bile reflux was confirmed as an independent risk factor for progression from precancerous lesions to cancer.

Conclusions: Bile reflux on endoscopy as well as age, dietary habits and a family history of GC were independent risk factors for the development of precancerous gastric lesions and GC.

KEYWORDS
bile reflux, gastric neoplasms, intestinal metaplasia, precancerous lesion

1 | INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies worldwide, ranking third for cancer-related mortality and fifth for incidence.[1] The development of intestinal GC has been proven to model the multistep process from normal gastric mucosa, through chronic gastritis (CG) (non-atrophic and atrophic gastritis), intestinal metaplasia (IM) and intraepithelial neoplasia (IN), to carcinoma.[2]

IM and IN are often considered to be precancerous lesions (PCL) that, without further intervention, will turn into early GC.[3] Patients with IM have a 6–9-fold higher incidence of progression to GC than those with normal mucosa.[4,5] Up to 23% of the cases with low-grade intraepithelial neoplasia (LGIN) may develop cancer while high-grade intraepithelial neoplasia (HGIN) has a even higher rate (60%-85%).[6]

In recent decades the mechanisms that lead to chronic inflammation and carcinoma have been explored. *Helicobacter pylori* (*H. pylori*)-induced inflammation has been proven to be closely associated with the occurrence, development, invasion and metastasis of GC.[7,8] Eradication of *H. pylori* alone leads to a decrease in relative risk from 34% to 77% in the development of noncardia GC. However, further stratified analyses have demonstrated that *H. pylori* eradication decreased the risk of IM development but not of that of dysplasia.[9,10] This suggests that other factors may induce chronic inflammation as well, leading to GC progression.

Bile reflux gastritis, also known as biliary gastritis or duodenogastric reflux, refers to the retrograde flow of bile into the stomach.[11] Based on epidemiological studies, in vitro experiments and animal models, bile reflux is strongly associated with cardiac IM and Barrett's esophagus (BE).[12-15] Bile reflux is also a primary risk factor for the occurrence of remnant GC after surgery for benign diseases.[16] However, the relationship between endoscopic bile reflux and precancerous gastric lesions and GC remains unclear.

Therefore, we designed this cross-sectional study to identify whether endoscopic bile reflux and other factors are independent risk factors for precancerous gastric lesions and GC.

2 | PARTICIPANTS AND METHODS

2.1 | Study design and population

We conducted a multicenter, cross-sectional study among patients with upper gastrointestinal symptoms and indications for an upper endoscopy in five hospitals in China (Xijing Hospital [Xi'an, Shaanxi Province], Shaanxi Traditional Chinese Medicine Hospital [Xi'an, Shaanxi Province], the First Affiliated Hospital of Zhengzhou University [Zhengzhou, Henan Province], Affiliated Hospital of Qinghai University [Xining, Qinghai Province], and Mianyang Central Hospital [Mianyang, Sichuan Province]) from June to October, 2019. These hospitals, from four provinces in the central and western regions of China at each level of local healthcare, were selected using a convenience sampling method. The study was approved by the Ethics Committees of each participating hospital and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from each participant prior to the trial registration at clinicaltrials.gov (no. NCT03976739).

Participants of both sexes who met the following inclusion criteria were eligible for enrollment in the study: (a) aged between 18 and 75 years; (b) with upper gastrointestinal symptoms and indications for an upper endoscopy; (c) willing to undergo test for *H. pylori* diagnosis; and (d) agreed to undergo upper endoscopy and biopsy. Exclusion criteria were as follows: (a) previous upper gastrointestinal surgeries; (b) previous diagnosis of malignancy; (c) contraindications to gastroscopy and biopsy; (d) could not be interviewed due to major central nervous system disease or mental disorder, etc; (e) pregnant or lactating; and (f) those who refused to give their informed consent. All enrolled participants were further grouped according to their histopathologic reports.

Biopsy specimens from cancer mass and adjacent tissues (according to the condition under the upper endoscopy) were obtained from the participants who were diagnosed with GC under endoscopy. Other participants underwent at least five biopsies as recommended by the Sydney system, including two from the greater and lesser curvature of the corpus, one from the incisura and two from the greater and lesser curvature of the antrum, respectively.[17]

2.2 | Grades of bile reflux

The bile reflux grading criteria used in the current study were consistent with the retrospective study we previously conducted.[18] According to the color and status of the mucous lake under endoscopy, the grade of bile reflux was determined as follows: (a) no bile reflux, clear or no mucous lake; (b) grade I, light yellow mucous lake; (c) grade II, yellowish–green mucous lake; and (d) grade III, dark yellow, turbid and viscous mucous lake with bile patches.[18]

2.3 | Histopathological findings and diagnosis of *H. pylori* infection

All the participants were divided into three groups based on their histopathologic results: (a) the CG group, including CG with or without atrophy; (b) the PCL group, including gastric IM and LGIN; and (c) the GC group, including HGIN and GC. Two experienced gastrointestinal pathologists who were blinded to the results of upper endoscopy and the questionnaires (as mentioned below) seperately determined and confirmed the severity of the lesion. Disagreement over the pathological results was resolved by discussion with a third pathologist.

Current *H. pylori* infection was confirmed by a ^{13}C-urea breath test (UBT) and the technician who performed the breath test was blinded to the endoscopic finding, histopathological result and

questionnaire.[13,14] The current *H. pylori* status and history of *H. pylori* infection of the participants were systematically recorded.

2.4 | Study questionnaire and baseline characteristics

All participants were interviewed by professional assistants in each center. The following information was obtained by a questionnaire from each participant before the endoscopic examination: their sex, age, height, bodyweight, body mass index (BMI), ethnicity, history of alcohol consumption and smoking, place of residence, education level, blood type, occupation, type of work, source of drinking water, familial history of GC, history of drug use (especially nonsteroidal anti-inflammatory drugs [NSAIDs]), *H. pylori* infection status, dietary habits (high-salt diet, leftover dishes, fried, preserved, spicy or smoked food, fresh fruits and fresh vegetables), and habits of tea and coffee drinking.

2.5 | Statistical analysis

All the statistical analysis were performed by using the SPSS Statistics 23.0 (IBM, Armonk, NY). Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as numbers and percentages or frequencies. The comparison of differences was performed using the Student's *t*-test or one-way ANOVA for continuous variables, and using the Fisher's exact test or χ^2 test for categorical variables. A multivariate logistic regression model was used to calculate the odds ratio (OR) and their 95% confidence interval (CI) to estimate the relationship between bile reflux and precancerous gastric lesions or GC and, to identify the associated independent risk factors. Variables that were statistically significant in the univariate analysis as well as those had been reported to have an impact on the occurrence and development of GC were included in the multivariate analysis. Three single multivariable models were used to compare the independent risk factors between each of the two groups. To analyze the risk factors related to different types of PCL we compared IM, LGIN and HGIN with CG. A two-tailed *P* value of less than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics of the participants

From June 2019 to October 2019 a total of 1162 patients were recruited from the Endoscopic Center of the five hospitals. GC was detected in 7.1% (n = 83) of all participants, and 35.4% (n = 411) were diagnosed with PCL. Baseline characteristics of the participants in the three groups are summarized in Table 1. The PCL and GC groups were significantly older than the CG group. Most participants with PLC and GC were men (both *P* < 0 .001), while the proportion of men and women was comparable in the CG group. The GC group had a lower BMI (21.8 ± 3.3 kg/m^2) than the PCL (23.0 ± 3.8 kg/m^2) and CG (22.7 ± 3.6 kg/m^2) groups (*P* = 0.029). In addition, the PCL and GC groups were more likely to report a family history of GC was higher in the PCL and GC groups than the CG group (16.1% and 18.1% vs 8.7%), and most participants with GC were rural residents and had a lower educational level (all *P* < 0.001). Although there were no statistically significant differences in ethnicity, blood type or *H. pylori* infection status among the three groups, obvious differences in smoking and alcohol status, water source and detection rate of bile reflux were observed.

The PCL group included 379 patients with IM and 32 with LGIN. We compared the factors between the two subgroups, as shown in Table S1. There were more smokers and alcohol drinkers in the LGIN subgroup, while other factors remained insignificant.

3.2 | Multivariate analysis

Multivariate logistic regression analysis showed that participant's age, history of smoking, drinking of non-tap water, a family history of GC, and a high-salt diet, consumption of pickled food and bile reflux were independent risk factors for PCL and GC. OR and 95% CI with and without *H. pylori* adjustment are presented in Table S2. Based on the the multivariate logistic regression analysis three multivariate analysis models were conducted to compare the difference in variables between each pair of the groups among all three groups. As shown in Table 2, we compared the 668 patients in the CG group and 411 patients in the PCL group using multivariate logistic regression. The results demonstrated independent hazard associations of age, sex, water source, family history of GC, overnight consumption of dishes, pickled food and bile reflux grade with PCL, with or without adjustment for *H. pylori*. Moreover, an increasing OR were observed with a higher grade of bile reflux. However, sufficient consumption of fresh vegetables and fruits (adjusted OR 0.58 and 0.65, respectively) was observed to be relatively protective against PCL.

Another logistic regression model was performed to compare the CG and the GC groups to determine the independent risk factors for GC. As shown in Table 3, age (adjusted OR 1.10), education level (adjusted OR 3.01), residence (rural: adjusted OR 1.94), a family history of GC (adjusted OR 3.08), consumption of a high-salt diet (adjusted OR 2.42), spicy food (adjusted OR 3.15) and the presence of bile reflux (adjusted OR 1.94) were found to be independent risk factors for GC when compared with relatively mild lesions.

We further used a logistic regression model to determine whether any factor plays a role in the progression from the severe mucosal lesion to the final stage of carcinoma, and found that age (adjusted OR 1.08), residence (rural: adjusted OR 2.62), over-consumption of spicy food (adjusted OR 3.10) and bile reflux (adjusted OR 1.75) were independent risk factors for GC (Table 4).

TABLE 1 Baseline characteristics of the participants among the chronic gastritis (CG), precancerous lesion (PCL), and the gastric cancer (GC) groups

	CG (n = 668)	PCL (n = 411)	GC (n = 83)	P value
Age, y (mean ± SD)	47.2 ± 12.5	52.5 ± 9.7[a]	59.5 ± 10.1[a,b]	<0.001
Sex, n (%)				<0.001
Male	332 (49.7)	245 (59.6)[a]	56 (67.5)[a]	
Female	336 (50.3)	166 (40.4)	27 (32.5)	
Ethnicity, n (%)				0.085
Han	617 (92.4)	393 (95.6)	76 (91.6)	
Others	51 (7.6)	18 (4.4)	7 (8.4)	
BMI, kg/m^2 (mean ± SD)	22.7 ± 3.6	23.0 ± 3.8	21.8 ± 3.3[a,b]	0.029
Residence, n (%)				<0.001
Urban	387 (57.9)	249 (60.6)	24 (28.9)[a,b]	
Town	98 (14.7)	57 (13.9)	12 (14.5)	
Rural	183 (27.4)	105 (25.5)	47 (56.6)[a,b]	
Education level, n (%)				<0.001
High (bachelor's degree or above)	259 (38.8)	122 (29.7)[a]	6 (7.2)[a,b]	
Low (below bachelor's degree)	409 (61.2)	289 (70.3)[a]	77 (92.8)[a,b]	
Smoking, n (%)				<0.001
Current smoker	144 (21.5)	110 (26.8)	34 (41.0)[a,b]	
Former smoker	58 (8.7)	56 (13.6)[a]	12 (14.5)[a,b]	
Never	466 (69.8)	245 (59.6)[a]	37 (44.6)[a,b]	
Alcohol consumption, n (%)				0.018
Current drinker	100 (15.0)	65 (15.8)	13 (15.7)	
Former drinker	51 (7.6)	50 (12.2)[a]	2 (2.4)	
Never	517 (77.4)	296 (72.0)[a]	68 (81.9)[b]	
Water source, n (%)				<0.001
Tap water	597 (89.4)	332 (80.8)[a]	55 (66.3)[a,b]	
Well water	61 (9.1)	56 (13.6)[a]	25 (30.1)[a,b]	
River and lake	10 (1.5)	23 (5.6)[a]	3 (3.6)	
Family history of GC, n (%)	58 (8.7)	66 (16.1)[a]	15 (18.1)[a]	<0.001
History of drug use, n (%)				
NSAIDs	8 (1.2)	3 (0.7)	2 (2.4)	0.246
Metformin	16 (2.4)	11 (2.7)	2 (2.4)	0.958
Statin	3 (0.4)	5 (1.2)	1 (1.2)	0.643
Diet (usually: >thrice/wk), n (%)				
High-salt diet	156 (23.4)	113 (27.5)	36 (43.4)[a,b]	<0.001
Leftover dishes	211 (31.6)	185 (45.0)[a]	50 (60.2)[a,b]	<0.001
Fried food	195 (29.2)	119 (29.0)	37 (44.6)[a,b]	0.013
Pickled food	168 (25.1)	140 (34.1)[a]	46 (55.4)[a,b]	<0.001
Spicy food	340 (50.9)	202 (49.1)	64 (77.1)[a,b]	<0.001
Smoke-dried food	76 (11.4)	42 (10.2)	3 (3.6)	0.077
Fresh vegetable consumption (>100 g/d)	155 (23.2)	128 (31.1)[a]	17 (20.5)	0.01
Fresh fruit consumption (>100 g/d)	218 (32.6)	160 (38.9)[a]	31 (37.3)	0.102
Beverage, n (%)				
Tea	206 (30.8)	114 (27.7)	30 (36.1)	0.259
Coffee	23 (3.4)	9 (2.2)	1 (1.2)	0.314
Blood type, n (%)				0.147
A	81 (12.1)	59 (14.4)	6 (7.2)	

(Continues)

TABLE 1 (Continued)

	CG (n = 668)	PCL (n = 411)	GC (n = 83)	P value
B	112 (16.8)	57 (13.9)	7 (8.4)	
O	109 (16.3)	79 (19.2)	17 (20.5)	
AB	45 (6.7)	33 (8.0)	5 (6.0)	
Unknown/missing	321 (48.1)	183 (44.5)	48 (57.8)	
H. pylori status, n (%)				0.759
Current infection	234 (35.0)	136 (33.1)	25 (30.1)	
Former infection	110 (16.5)	63 (15.3)	16 (19.3)	
Never infected	324 (48.5)	212 (51.6)	42 (50.6)	
Bile reflux, n (%)				<0.001
No	469 (70.2)	246 (59.9)	39 (47.0)	
Yes	199 (29.8)	165 (40.1)[a]	44 (53.0)[a,b]	
Grade I	104 (15.6)	72 (17.5)	21 (25.3)[a]	
Grade II	69 (10.3)	61 (14.8)[a]	12 (14.5)	
Grade III	26 (3.9)	32 (7.8)[a]	11 (13.3)[a]	

Note: P < 0.05 compared with the CG group,[a] or with the PCL group.[b]
The CG group includes non-atrophic gastritis and chronic atrophic gastritis. The PCL group includes gastric intestinal metaplasia and mild-to-moderate dysplasia. And the GC group includes severe intraepithelial neoplasia and gastric cancer.
Abbreviations: BMI, body mass index; *H. pylori*, *Helicobacter pylori*; NSAIDs, nonsteroidal anti-inflammatory drugs; SD, standard deviation.

TABLE 2 Multivariate logistic regression analysis model comparing the chronic gastritis group and the precancerous lesion group

	Univariate analysis		Multivariate analysis			
			Unadjusted		*H. pylori*-adjusted	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.04 (1.03–1.05)	<0.05	1.05 (1.03–1.06)	<0.05	1.05 (1.03–1.06)	<0.05
Sex (ref: female)	1.49 (1.17–1.92)	<0.001	1.65 (1.15–2.37)	<0.001	1.67 (1.16–2.39)	<0.001
BMI	1.02 (0.99–1.05)	>0.05	1.03 (0.99–1.07)	>0.05	1.03 (0.99–1.07)	>0.05
Education level (ref: high)	1.50 (1.15–1.95)	<0.05	1.07 (0.79–1.45)	>0.05	1.08 (0.79–1.47)	>0.05
Smoking status (ref: never)						
Current smoker	1.45 (1.09–1.95)	<0.05	1.10 (0.74–1.54)	>0.05	1.11 (0.75–1.65)	>0.05
Former smoker	1.84 (1.22–2.74)	<0.05	1.19 (0.71–1.99)	>0.05	1.18 (0.70–1.98)	>0.05
Alcohol status (ref: never)						
Current drinker	1.14 (0.81–1.60)	>0.05	0.97 (0.63–1.48)	>0.05	0.96 (0.63–1.47)	>0.05
Former drinker	1.71 (1.13–2.59)	<0.05	1.28 (0.76–2.13)	>0.05	1.26 (0.76–2.12)	>0.05
Water source (ref: tap)						
Well	1.65 (1.12–2.43)	<0.05	1.28 (0.84–1.95)	>0.05	1.26 (0.82–1.93)	>0.05
River and lake	4.14 (1.95–8.79)	<0.001	4.03 (1.82–8.90)	<0.001	4.19 (1.89–9.31)	<0.001
Family history of GC (ref: no)	2.01 (1.38–2.93)	<0.001	1.99 (1.33–2.98)	<0.001	2.01 (1.34–3.02)	<0.001
Leftover dishes (ref: no)	1.77 (1.38–2.29)	<0.05	1.40 (1.04–1.87)	<0.05	1.41 (1.05–1.88)	<0.05
Pickled food (ref: no)	1.54 (1.18–2.01)	<0.05	1.19 (0.88–1.62)	>0.05	1.20 (0.89–1.63)	0.05
Fresh vegetable consumption (ref: <100 g/d)	1.48 (1.13–1.96)	<0.05	0.59 (0.43–0.79)	<0.05	0.58 (0.43–0.79)	<0.05
Fresh fruit consumption (ref: <100 g/d)	1.10 (1.01–1.19)	<0.05	0.67 (0.50–0.88)	<0.05	0.65 (0.49–0.87)	<0.05
Grade of bile reflux (ref: no)						
I	1.32 (0.94–1.85)	<0.001	1.61 (1.11–2.32)	<0.001	1.61 (1.12–2.33)	<0.001
II	1.69 (1.16–2.46)	<0.001	2.07 (1.37–3.13)	<0.001	2.05 (1.36–3.11)	<0.001
III	2.35 (1.37–4.03)	<0.001	2.84 (1.55–5.20)	<0.001	2.79 (1.52–5.11)	<0.001

Abbreviations: BMI, body mass index; CI, confidence interval; GC, gastric cancer; *H. pylori*, *Helicobacter pylori*; OR, odds ratio.

TABLE 3 Multivariate logistic regression analysis model comparing the chronic gastritis group and the gastric cancer (GC) group

| | Univariate analysis | | Multivariate analysis | | | |
| | | | Unadjusted | | *H. pylori*-adjusted | |
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.10 (1.08–1.13)	<0.001	1.10 (1.07–1.13)	<0.001	1.10 (1.07–1.13)	<0.001
Sex (ref: female)	2.10 (1.29–3.41)	0.014	1.81 (0.79–4.13)	>0.05	1.92 (0.84–4.41)	>0.05
BMI	0.93 (0.86–0.99)	0.041	0.92 (0.84–1.01)	>0.05	0.92 (0.85–1.01)	>0.05
Education level (ref: high)	8.13 (3.49–18.92)	<0.001	2.91 (1.14–7.46)	<0.001	3.01 (1.17–7.76)	<0.001
Residence (ref: urban)						
Town	1.97 (0.95–4.09)	>0.05	1.38 (0.57–3.38)	>0.05	1.35 (0.55–3.31)	>0.05
Rural	4.14 (2.46–6.98)	<0.001	1.90 (0.99–3.64)	>0.05	1.94 (1.01–3.74)	<0.05
History of smoking (ref: never)	2.87 (1.81–4.56)	<0.001	2.03 (0.90–4.62)	>0.05	1.97 (0.87–4.47)	>0.05
History of alcohol consumption (ref: never)	0.755 (0.42–1.36)	>0.05	0.72 (0.32–1.60)	>0.05	0.69 (0.31–1.54)	>0.05
Water source (ref: tap water)						
Non-tap water	1.57 (0.82–3.03)	>0.05	0.96 (0.18–5.01)	>0.05	0.89 (0.17–4.60)	>0.05
Family history of GC (ref: no)	2.32 (1.25–4.32)	<0.001	2.88 (1.30–6.38)	<0.001	3.08 (1.38–6.87)	<0.001
High-salt diet (ref: no)	2.51 (1.57–4.02)	<0.001	2.35 (1.31–4.24)	<0.001	2.42 (1.33–4.38)	<0.001
Leftover dishes (ref: no)	3.28 (2.05–5.25)	<0.001	1.35 (0.74–2.45)	>0.05	1.41 (0.77–2.59)	>0.05
Fried food (ref: no)	1.95 (1.23–3.10)	<0.05	1.31 (0.70–2.47)	>0.05	1.37 (0.72–2.58)	>0.05
Pickled food (ref: no)	3.70 (2.32–5.90)	<0.001	1.21 (0.65–2.25)	>0.05	1.19 (0.64–2.21)	>0.05
Spicy food (ref: no)	3.25 (1.91–5.54)	<0.001	3.12 (1.65–5.93)	<0.001	3.15 (1.65–5.99)	<0.001
Bile reflux (ref: no)	2.659 (1.68–4.22)	<0.001	1.91 (1.08–3.40)	<0.001	1.94 (1.09–3.46)	<0.001

Abbreviations: BMI, body mass index; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; OR, odds ratio.

TABLE 4 Multivariate logistic regression analysis model comparing the precancerous lesion group and the gastric cancer (GC) group

| | Univariate analysis | | Multivariate analysis | | | |
| | | | Unadjusted | | *H. pylori*-adjusted | |
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.08 (1.05–1.11)	<0.05	1.08 (1.04–1.11)	<0.05	1.08 (1.04–1.11)	<0.05
Sex (ref: female)	1.41 (0.85–2.32)	>0.05	1.40 (0.64–3.09)	>0.05	1.37 (0.62–3.03)	>0.05
BMI	0.91 (0.85–098)	<0.05	0.93 (0.85–1.01)	>0.05	0.93 (0.85,1.01)	>0.05
Education level (ref: high)	5.42 (2.30–12.77)	<0.05	2.22 (0.85–5.25)	>0.05	2.28 (0.87–5.98)	>0.05
Residence (ref: urban)						
Town	2.36 (1.10–5.05)	<0.05	1.69 (0.71–4.00)	>0.05	1.68 (0.71–4.00)	>0.05
Rural	5.03 (2.88–8.76)	<0.05	2.62 (1.31–5.25)	<0.05	2.62 (1.31–5.24)	<0.05
History of smoking (ref: never)	1.84 (1.14–2.95)	<0.05	1.37 (0.63–2.99)	>0.05	1.43 (0.65–3.14)	>0.05
History of alcohol consumption (ref: never)	0.57 (0.31–1.03)	>0.05	0.50 (0.24–1.06)	>0.05	0.49 (0.23–1.05)	>0.05
Water source (ref: tap water)						
Non-tap water	2.14 (1.28–3.59)	<0.05	1.25 (0.63–2.45)	>0.05	1.24 (0.63–2.44)	>0.0
Family history of GC (ref: no)	1.15 (0.62–2.14)	>0.05	1.55 (0.75–3.21)	>0.05	1.55 (0.74–3.22)	>0.05
High-salt diet (ref: No)	2.02 (1.24–3.28)	<0.05	1.25 (0.70–2.21)	>0.05	1.22 (0.69–2.18)	>0.05
Leftover dishes (ref: no)	1.85 (1.15–2.99)	<0.05	0.86 (0.47–1.56)	>0.05	0.85 (0.47–1.56)	>0.05
Fried food (ref: no)	1.97 (1.22–3.21)	<0.05	0.80 (0.44–1.48)	>0.05	0.80 (0.43–1.48)	>0.05
Pickled food (ref: no)	2.41 (1.49–3.88)	<0.05	1.42 (0.78–2.61)	>0.05	1.42 (0.77–2.60)	>0.05
Spicy food (ref: no)	3.49 (2.02,6.03)	<0.001	3.12 (1.63–5.98)	<0.001	3.10 (1.61–5.93)	<0.001
Bile reflux (ref: no)	1.68 (1.05–2.70)	<0.001	1.76 (1.02–3.03)	<0.001	1.75 (1.01–3.01)	<0.001

Abbreviations: BMI, body mass index; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; OR, odds ratio.

4 | DISCUSSION

The results of our study reveal the factors that may have a strong association with the development and progression of precancerous gastric lesions and GC. Our study was a cross-sectional study conducted at multiple centers and a detailed questionnaire was designed to collect comprehensive demographic information and dietary habits of the participants. All of the participants we recruited underwent upper endoscopy and mapping biopsies. Through systematic analysis, we assessed the factors that might be associated with PCL, and identified those that might influence the progression from precancerous conditions to carcinoma.

To the best of our knowledge this was the first time that bile reflux was confirmed as an independent factor associated with precancerous gastric lesions and GC. The explanation for these observations derives from two phenomena: (a) the incidence of primary bile reflux has been gradually increasing during the past few decades;[11] and (b) the influence of primary bile reflux remains unclear, which results in its neglect by gastroenterologists, endoscopists and pathologists. However, it may play a crucial role in the progression from normal gastric mucosa through PCL to GC in the absence of *H. pylori* infection. A positive link between bile reflux and a subsequent elevated risk of IM and GC development has been consistently reported by two Japanese clinical studies.[19,20]

A number of experiments have demonstrated the underlying mechanisms by which bile acid contributes to the progression of GC and PCL. First, hydrophobic bile acids such as chenodeoxycholic acid can cause increased cellular invasion of gastric cells through the activation of PKC and COX-2 induction.[21] In addition, the bile acid receptor TGR5 plays a role in promoting the epithelial–mesenchymal transition of GC cells.[22] Our group has previously demonstrated that the miR-92a-1-5p/FOXD1/NF-κB/CDX2 regulatory axis plays a crucial role in the development of IM caused by bile acid.[23] As a result, more attention should be paid to bile reflux during endoscopy.

In the present study we found that a family history of GC may be an important factor linked to the occurrence of precancerous gastric lesions and GC, with OR reaching 2 to 3, which is consistent with the results of previous epidemiological trials. Previous studies have reported that a family history of any precancerous changes and GC presented a 2.5-fold and a 3.8-fold hazard, respectively, of noncardia GC compared with index people who had relatives with mild mucosal changes.[24] Recently, researchers found that individuals with a family history of GC were at a high risk of IN or dysplasia.[25] In addition, such patients were more likely to progress from PCL to GC.[26] This suggests that a family history of GC is a significant risk factor for the development of GC and the progression of PCL to GC. Therefore, when screening for GC close attention should be paid to the family history of GC.

Moreover, some specific dietary factors seem to be associated with the development of malignant mucosal lesions. In our study, high-salt diet and an excess of spicy food may be important dietary factors related to the occurrencce of GC, whereas fresh vegetables and fruits may play a protective role. Several trials focused on the impact of routine dietary intake on GC have made similar observations, and high-salt intake has been reported to be associated with an increased risk of atrophic gastritis with IM.[27] The results of a 10-year epidemiological study of GC also demonstrated that a high-salt, high-fat and spicy diet was a significant factor for GC in both female and male participants.[28] A high-salt diet can lead to damage and a persistent inflammatory state of the gastric mucosa and increase the incidence of endogenous mutations. It is also thought to contribute to the colonization by *H. pylori*, and to have a synergistic effect between salt intake and *H. pylori* infection in the development of GC.[29,30] Although current and previous studies suggest that a high intake of spicy food may be associated with an increased incidence of cancer[31] this view remains controversial. This association may be related to different populations, regions, living habits and environmental factors. Our results are in accordance with the notion that a familial aggregation of GC and gastric precancerous changes could be due to a genetic or inherited predisposition and exposure to similar environmental factors, such as carcinogenic *H. pylori* strains (if any), common dietary habits or other carcinogenic exposures, within a family.[28]

Unexpectedly, our study was unable to demonstrate a statistical association between *H. pylori* status and both premalignant lesions and GC, which conflicts with what is well known according to public consensus and what has been reported in earlier trials.[32] We further reviewed some participants to determine the underlying reason. Owing to the increasing awareness of *H. pylori* infection, a proportion of participants from the CG group asked for a gastroscopy after knowing their *H. pylori* infection status.

Interestingly, discriminatory differences in the distribution of educational level and residence were demonstrated in the present study. The proportion of participants with a low education level and residence in the rural area was much higher in the GC group than in the other two groups and this result was in line with a national epidemiological report in 2015,[33] and may be explained by the lack of *H. pylori* eradication treatment and regular endoscopic surveillance in these participants. In addition, there is a strong association between drinking unchlorinated water from wells and surface water sources and the risk of GC.[34]

The development of GC is a complex pathological process with multifactorial involvement and multiple steps. Our results found discriminatory associations with older age, consumption of spicy food and bile acid for precancerous gastric conditions and similar associations were also presented through comparisons with the GC group. When comparing the PCL group with the GC group, the abovementioned risk factors still showed elevated OR, which also indicates that the occurrence of GC is the result of a continuous effect of each risk factor. A previous cross-sectional study performed in a U.S. population reported strong associations of older age, male sex, nonwhite race or ethnicity, and current smoking with gastric IM as well as atrophic gastritis.[35] However, this study was derived from a U.S. veteran cohort with an imbalanced sex and racial distribution and was unable to collect important relevant information, such as previous *H. pylori* infection and routine diet, and particularly the grade of bile reflux. A discriminatory strength of our study is that we enrolled

participants who were diagnosed with all histological types of gastric mucosa lesions, including GC.

Primary bile reflux, which occurs without gastric surgery, is associated with gallbladder dysfunction and gastric or duodenal motility disorders.[11] The results of our previous retrospective study showed that bile reflux was associated with age, sex and duration of fasting,[18] and there have been no reports of bile reflux being associated with other factors, such as water source, family history of GC, a high-salt diet, spicy food and place of residence. Therefore, we identified bile reflux as one of the independent risk factors for GC and PCL.

There were some limitations to the present study. First, all the participants were recruited from endoscopy centers in hospitals, which implies that the participants we enrolled were symptomatic with an indication for upper endoscopy; thus, minimal selection bias was inevitable and a higher prevalence of PCL and GC than average was observed in our study. Our study was also limited by the characteristics of cross-sectional studies. Accordingly, we were unable to observe prospectively the deterioration or improvement of participants with different stages of mucosa lesions, and an observational follow-up study that requires regular surveillance for participants is necessary to construct a predictive model for risk stratification. Additionally, long-term multicenter clinical trials should be performed to identify the role of bile reflux in the whole progression from normal mucosa to gastric malignancy. Although we have described the minor difference between subgroups of the precancerous group, owing to the unsatisfactory proportion of the two subgroups (IM: LGIN = 10:1), the results we obtained were relatively unconvincing.

In conclusion, bile reflux under endoscopy, age, family history of GC and some specific dietary habits are independent risk factors for the occurrence of precancerous gastric lesions and GC.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Lu Yao Zhang https://orcid.org/0000-0001-9408-9496
Dan Li https://orcid.org/0000-0003-4770-1034
Yong Quan Shi https://orcid.org/0000-0001-9515-7577

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424.

2. Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. *Lancet.* 1975;2(7924):58-60.

3. Lage J, Uedo N, Dinis-Ribeiro M, Yao K. Surveillance of patients with gastric precancerous conditions. *Best Pract Res Clin Gastroenterol.* 2016;30(6):913-922.

4. Song H, Ekheden IG, Zheng Z, Ericsson J, Nyrén O, Ye W. Incidence of gastric cancer among patients with gastric precancerous lesions: observational cohort study in a low risk Western population. *BMJ.* 2015;351:h3867. https://doi.org/10.1136/bmj.h3867.

5. Choi AY, Strate LL, Fix MC, et al. Association of gastric intestinal metaplasia and East Asian ethnicity with the risk of gastric adenocarcinoma in a U.S. population. *Gastrointest Endosc.* 2018;87(4):1023-1028.

6. Dinis-Ribeiro M, Areia M, de Vries AC, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy.* 2012;44(1):74-94.

7. Amieva M, Peek RM Jr. Pathobiology of *Helicobacter pylori*-induced gastric cancer. *Gastroenterology.* 2016;150(1):64-78.

8. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. *J Cancer Prev.* 2015;20(1):25-40.

9. Lee YC, Chiang TH, Chou CK, et al. Association between *Helicobacter pylori* eradication and gastric cancer incidence: a systematic review and meta-analysis. *Gastroenterology.* 2016;150(5):1113-1124.e5.

10. Duan F, Song C, Zhang J, et al. Evaluation of the epidemiologic efficacy of eradicating *Helicobacter pylori* on development of gastric cancer. *Epidemiol Rev.* 2019;41(1):97-108.

11. McCabe ME IV, Dilly CK. New causes for the old problem of bile reflux gastritis. *Clin Gastroenterol Hepatol.* 2018;16(9):1389-1392.

12. Malfertheiner P, Peitz U. The interplay between *Helicobacter pylori*, gastro-oesophageal reflux disease, and intestinal metaplasia. *Gut.* 2005;54(Suppl):i13-i20.

13. Souza RF. The role of acid and bile reflux in oesophagitis and Barrett's metaplasia. *Biochem Soc Trans.* 2010;38(2):348-352.

14. Kazumori H, Ishihara S, Takahashi Y, Amano Y, Kinoshita Y. Roles of Krüppel-like factor 4 in oesophageal epithelial cells in Barrett's epithelium development. *Gut.* 2011;60(5):608-617.

15. Das KM, Kong Y, Bajpai M, et al. Transformation of benign Barrett's epithelium by repeated acid and bile exposure over 65 weeks: a novel in vitro model. *Int J Cancer.* 2011;128(2):274-282.

16. Ohira M, Toyokawa T, Sakurai K, et al. Current status in remnant gastric cancer after distal gastrectomy. *World J Gastroenterol.* 2016;22(8):2424-2433.

17. Dixon MF, Genta RM, Yardley JH, Correa P; International Workshop on the Histopathology of Gastritis, Houston 1994. Classification and grading of gastritis. The updated Sydney system. *Am J Surg Pathol.* 1996;20(10):1161-1181.

18. Li D, Zhang J, Yao WZ, et al. The relationship between gastric cancer, its precancerous lesions and bile reflux: a retrospective study. *J Dig Dis.* 2020;21(4):222-229.

19. Matsuhisa T, Arakawa T, Watanabe T, et al. Relation between bile acid reflux into the stomach and the risk of atrophic gastritis and intestinal metaplasia: a multicenter study of 2283 cases. *Dig Endosc.* 2013;25(5):519-525.

20. Tatsugami M, Ito M, Tanaka S, et al. Bile acid promotes intestinal metaplasia and gastric carcinogenesis. *Cancer Epidemiol Biomarkers Prev.* 2012;21(11):2101-2107.

21. Wu YC, Chiu CF, Hsueh CT, Hsueh CT. The role of bile acids in cellular invasiveness of gastric cancer. *Cancer Cell Int.* 2018;18:75. https://doi.org/10.1186/s12935-018-0569-0.

22. Carino A, Graziosi L, D'Amore C, et al. The bile acid receptor GPBAR1 (TGR5) is expressed in human gastric cancers and promotes

epithelial–mesenchymal transition in gastric cancer cell lines. *Oncotarget*. 2016;7(38):61021-61035.

23. Li T, Guo H, Li H, et al. MicroRNA-92a-1-5p increases CDX2 by targeting FOXD1 in bile acids-induced gastric intestinal metaplasia. *Gut*. 2019;68(10):1751-1763.

24. Song H, Ekheden IG, Ploner A, Ericsson J, Nyren O, Ye W. Family history of gastric mucosal abnormality and the risk of gastric cancer: a population-based observational study. *Int J Epidemiol*. 2018;47(2):440-449.

25. Wu R, Yang C, Ji L, Fan ZN, Tao YW, Zhan Q. Prevalence of gastric cancer precursors in gastroscopy-screened adults by family history of gastric cancer and of cancers other than gastric. *BMC Cancer*. 2020;20(1):1110. https://doi.org/10.1186/s12885-020-07612-8.

26. González CA, Pardo ML, Liso JMR, et al. Gastric cancer occurrence in preoplastic lesions: a long-term follow-up in a high-risk area in Spain. *Int J Cancer*. 2010;127(11):2654-2660.

27. Song JH, Kim YS, Heo NJ, et al. High salt intake is associated with atrophic gastritis with intestinal metaplasia. *Cancer Epidemiol Biomarkers Prev*. 2017;26(7):1133-1138.

28. Yan S, Li B, Bai ZZ, et al. Clinical epidemiology of gastric cancer in Hehuang valley of China: a 10-year epidemiological study of gastric cancer. *World J Gastroenterol*. 2014;20(30):10486-10494.

29. Furihata C, Ohta H, Katsuyama T. Cause and effect between concentration-dependent tissue damage and temporary cell proliferation in rat stomach mucosa by NaCl, a stomach tumor promoter. *Carcinogenesis*. 1996;17(3):401-406.

30. Caston RR, Loh JT, Voss BJ, et al. Effect of environmental salt concentration on the *Helicobacter pylori* exoproteome. *J Proteomics*. 2019;202:103374. https://doi.org/10.1016/j.jprot.2019.05.002.

31. Chen YH, Zou XN, Zheng TZ, et al. High spicy food intake and risk of cancer: a meta-analysis of case-control studies. *Chin Med J (Engl)*. 2017;130(18):2241-2250.

32. Wang F, Meng W, Wang B, Qiao L. *Helicobacter pylori*-induced gastric inflammation and gastric cancer. *Cancer Lett*. 2014;345(2):196-202.

33. Zheng RS, Sun KX, Zhang SW, et al. Report of cancer epidemiology in China, 2015. *Chin J Oncol*. 2019;41(1):19-28.(in Chinese).

34. Eichelberger L, Murphy G, Etemadi A, et al. Risk of gastric cancer by water source: evidence from the Golestan case-control study. *PLoS One*. 2015;10(5):e0128491. https://doi.org/10.1371/journal.pone.0128491.

35. Tan MC, Mallepally N, Liu Y, El-Serag HB, Thrift AP. Demographic and lifestyle risk factors for gastric intestinal metaplasia among US veterans. *Am J Gastroenterol*. 2020;115(3):381-387.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.