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• *BRIEF REPORTS* •

Effects of bile reflux on gastric mucosal lesions in patients with dyspepsia or chronic gastritis

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Abstract

AIM: To investigate the influences of bile reflux on profiles of gastric mucosal lesions in patients with dyspepsia or chronic gastritis.

METHODS: A total of 49 patients diagnosed with dyspepsia and chronic gastritis underwent 24-h ambulatory and simultaneous monitoring of intragastric bilirubin absorbance and pH values, and then they were divided into bile reflux positive group and bile reflux negative group. Severity of pathological changes in gastric mucosa including active inflammation, chronic inflammation, intestinal metaplasia, atrophy and dysplasia as well as *Helicobacter pylori* (*H pylori*) infection at the corpus, incisura and antrum were determined respectively according to update Sydney system criteria. The profiles of gastric mucosal lesions in the two groups were compared, and correlations between time-percentage of gastric bilirubin absorbance >0.14 and severity of gastric mucosal lesions as well as time-percentage of gastric pH >4 were analyzed respectively.

RESULTS: Thirty-eight patients (21 men and 17 women, mean age 44.2 years, range 25-61 years) were found existing with bile reflux (gastric bilirubin absorbance >0.14) and 11 patients (7 men and 4 women, mean age 46.2 years, range 29-54 years) were bile reflux negative. In dyspepsia patients with bile reflux, the mucosal lesions such as active inflammation, chronic inflammation, intestinal metaplasia, atrophy or *H pylori* infection in the whole stomach, especially in the corpus and incisura, were significantly more severe than those in dyspepsia patients without bile reflux. Moreover, the bile reflux time was well correlated with the severity of pathological changes of gastric mucosa as well as *H pylori* colonization in the near-end stomach, especially in the corpus region. No relevance was found between the time of bile reflux and pH >4 in gastric cavity.

CONCLUSION: Bile reflux contributes a lot to mucosal lesions in the whole stomach, may facilitate *H pylori* colonization in the corpus region, and has no influence on acid-exposing status of gastric mucosa in patients with dyspepsia or chronic gastritis.

Key words: Bile reflux; Chronic gastritis; Dyspepsia; *H pylori*; Gastric mucosa; Corpus

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INTRODUCTION

Reflux of bile and other contents of duodenum, along with gastric acid and *Helicobacter pylori* (*H pylori*) infection, are the main etiological factors which play roles in the pathophysiological processes leading to gastric mucosal lesions in patients with chronic gastritis, and to some extent, these factors may act synergistically[1,2]. However, the exact pathogenic features of bile reflux as well as its contributions to gastric mucosal lesions in this disease are still remaining unrevealed, *e.g.*, little is known about the relevance between bile reflux and profile of pathological changes of gastric mucosa, such as the locations and histological characteristics of these changes in chronic gastritis. In addition, the relationships between bile reflux and *H pylori* infection as well as the acid-exposing status of gastric mucosa are also not clear.

In the present study, by means of monitoring dynamically intragastric bilirubin absorbance, which reflects the status of bile reflux in stomach, along with the pH values in gastric cavity, we assessed the influences of bile reflux on severity of mucosal pathological changes, status of *H pylori* infection and the acid environment of mucosa in stomachs of patients with dyspepsia or chronic gastritis, with intention to shed new light on the exact role of bile reflux in the pathophysiological processes of chronic gastritis.

MATERIALS AND METHODS

Study subjects

We enrolled 49 consecutive patients who came to our out-patient department in the period of November 2000 to March 2002 (28 men and 21 women, mean age 44.5 years, range 25-61 years), all the patients had a diagnosis of chronic

gastritis or dyspepsia established by both gastroscopy and biopsy of gastric mucosa.

Assessment of profile of gastric mucosal lesions

In gastroscopy, five biopsies were taken from gastric mucosa in three different regions of the stomach according to the principles of update Sydney system[3], two from the antrum, 2-3 cm to pylora; two from the corpus, 8 cm to cardia, one in lesser curvature and the other in greater curvature; the fifth from the incisura. All the serial sections (5-μm thick) prepared from formalin-fixed, paraffin-embedded biopsy samples underwent hematoxylin and eosin staining (for conventional histological determination), AB-PAS (pH 2.5) staining (for evaluation of intestinal metaplasia) and modified Giemsa staining (for detection of *H pylori* infection). Each specimen was assessed for the grade of active inflammation, chronic inflammation, intestinal metaplasia, hyperplasia, atrophy, dysplasia and *H pylori* infection of gastric mucosa at corpus, incisura and antrum according to the criteria of update Sydney system (classified as +, ++ or +++, which were respectively given a score of 1, 2 or 3 in statistical processing; *H pylori* infection status was classified as negative or positive, which was given a score of 0 or 1 respectively in statistical processing).

Assessment of bile reflux and pH in gastric cavity

This work was performed in our clinic. Using a Bilitec 2000 bile reflux monitor and Synectics Digitrapper MKIII dynamic pH monitor respectively, all the patients underwent a 24-h ambulatory and simultaneous monitoring of bilirubin absorbance and pH in gastric cavity. Any drug that may inhibit gastric acid secretion, promote gastrointestinal motility, protect gastric mucosa or neutralize bile and antibiotics were stopped 2 wk before monitoring. In order to determine the lengths of esophagus and locations of lower esophageal sphincter (LES), esophagus pressure examination was conducted in all subjects before monitoring. The fiberoptic bilirubin probe and pH electrode were located 5 cm below LES inframargin. Nothing restricted patients' behavior, except ingesting diets free from substances with a similar absorption wavelength to that of bilirubin. The single-channeled, combined analysis of bile reflux and pH was conducted using Synectics analysis program. Patients with bilirubin absorbance >0.14 were regarded as bile reflux positive[4,5]. The time-percentages of bile reflux or pH >4 in gastric cavity were recorded respectively.

Statistics and ethical approval

The statistical differences in profiles of gastric mucosal lesions between bile reflux positive group and bile reflux negative group were determined using Student's *t* test. The prevalent rates of *H pylori* infection were compared using Fisher's exact test. The correlations between time-percentages of bile reflux and profiles of gastric mucosal lesions or time-percentages of pH >4 were analyzed using Pearson correlation analysis. All the statistical analyses were performed using the SAS statistical software package (SAS Institute Inc., Cary, NC, USA). *P* values <0.05 were considered statistically significant. Informed consent was obtained from all participants in concurrence with the Declaration of Helsinki.

RESULTS

Profiles of gastric mucosal lesions in patients with or without bile reflux

All the 49 patients fulfilled gastric bilirubin absorbance and pH monitoring. Among these patients, 38 (21 men and 17 women, average age 44.2 years, range 25-61 years) were found existing with intragastric bile reflux (bilirubin absorbance >0.14), whose mean time-percentage was 19.9±14.3% (range 5.02-61.57%). No bile reflux was found in the other 11 patients (7 men and 4 women), whose average age was 46.2 years (range 29-54 years). The bile reflux positive patients were well matched in age, sex, history and course with those without bile reflux.

The profiles of gastric mucosal lesions in dyspepsia patients with or without bile reflux are summarized in Table 1. The pathological grades of gastric mucosa in patients with bile reflux, e.g. active inflammation, chronic inflammation, intestinal metaplasia and atrophy in the near-end stomach (the corpus and incisura, $P<0.0001$-0.005), active inflammation and atrophy in the antrum ($P<0.05$), were significantly more severe than those in patients without bile reflux.

Profiles of H pylori infection in dyspepsia patients with or without bile reflux

The incidence of *H pylori* infection in the corpus of dyspepsia patients with bile reflux was significantly higher than that in the corpus of dyspepsia patients without bile reflux ($P<0.01$), while no significant differences were found in prevalence of *H pylori* infection in the incisura and antrum in these two groups. The overall *H pylori* infection rate [36.9% (14/38)] of bile reflux positive patients was lower than that [54.5% (6/11)] of patients without bile reflux, but with no statistical significance.

Correlation between bile reflux time and profile of gastric mucosal lesions in dyspepsia patients with bile reflux

The time-percentages of bile reflux in gastric cavity were well correlated with active inflammation ($r = 0.3949$, $P<0.05$), chronic inflammation ($r = 0.8938$, $P = 0.0001$), atrophy ($r = 0.4619$, $P<0.005$) and *H pylori* colonization ($r = 0.8938$, $P = 0.0001$) in the corpus. Similarly, chronic inflammation ($r = 0.6234$, $P = 0.0001$) and *H pylori* colonization ($r = 0.4992$, $P<0.005$) in the incisura were also found to be positively correlated with time-percentages of gastric bile reflux. No association was found between bile reflux time and gastric mucosal lesions in the antrum.

Correlation between time of bile reflux and pH >4 in gastric cavity in dyspepsia patients with bile reflux

In 38 dyspepsia patients with bile reflux, the time-percentages of bile reflux and pH >4 in gastric cavity were 19.9±14.3% and 25.4±16.7% respectively. No obvious correlation was found between the time of gastric bile reflux and pH >4.

DISCUSSION

Similar with the results of studies about remnant gastric mucosa after gastric operation[6,7], our study demonstrated that bile reflux positive patients with chronic gastritis had

Table 1 Profile of gastric mucosal lesions in dyspepsia patients with or without bile reflux, % (*n*)

	Antrum		Incisura		Corpus	
	BR+	BR–	BR+	BR–	BR+	BR–
Active gastritis	*P*<0.05		*P*<0.001		*P*<0.001	
Negative	13.1 (5/38)	0 (0/11)	50.0 (19/38)	54.5 (6/11)	39.5 (15/38)	72.7 (8/11)
+	39.5 (15/38)	63.6 (7/11)	15.8 (6/38)	45.5 (5/11)	42.1 (16/38)	27.2 (3/11)
++	34.2 (13/38)	18.2 (2/11)	28.9 (11/38)	0 (0/11)	15.8 (6/38)	0 (0/11)
+++	13.1 (5/38)	18.2 (2/11)	52.6 (2/38)	0 (0/11)	2.6 (1/38)	0 (0/11)
Chronic gastritis			*P*<0.001		*P*<0.001	
Negative	0 (38/38)	0 (0/11)	0 (38/38)	18.2 (2/11)	0 (38/38)	100 (11/11)
+	94.7 (36/38)	81.8 (9/11)	84.2 (32/38)	81.8 (9/11)	52.6 (20/38)	0 (0/11)
++	5.2 (2/38)	18.2 (2/11)	10.5 (4/38)	0 (0/11)	42.1 (16/38)	0 (0/11)
++	0 (0/38)	0 (0/11)	5.2 (2/38)	0 (0/11)	7.9 (3/38)	0 (0/11)
Intestinal metaplasia			*P*<0.001		*P*<0.001	
Negative	0 (38/38)	0 (0/11)	0 (38/38)	63.6 (7/11)	23.7 (9/38)	100 (11/11)
+	84.2 (32/38)	72.7 (8/11)	100 (38/38)	36.4 (4/11)	55.3 (21/38)	0 (0/11)
++	15.8 (6/38)	27.3 (3/11)	0 (0/38)	0 (0/11)	15.8 (6/38)	0 (0/11)
++	0 (0/38)	0 (0/11)	0 (0/38)	0 (0/11)	5.3 (2/38)	0 (0/11)
Atrophy	*P*<0.05		*P*<0.001		*P*<0.001	
Negative	0 (38/38)	0 (11/11)	18.4 (7/38)	63.6 (7/11)	21.1 (8/38)	100 (11/11)
+	78.9 (30/38)	72.7 (8/11)	73.6 (28/38)	27.3 (3/11)	71.1 (27/38)	0 (0/11)
++	21.0(8/38)	18.2 (2/11)	7.9 (3/38)	0 (0/11)	7.9 (3/38)	0 (0/11)
++	0 (0/38)	9.0 (1/11)	0 (0/38)	0 (0/11)	0 (0/38)	0 (0/11)
Dysplasia						
Negative	89.5 (34/38)	100 (11/11)	95.8 (36/38)	100 (11/11)	86.8 (33/38)	100 (11/11)
+	10.5 (4/38)	0 (0/11)	7.9 (3/38)	0 (0/11)	13.2 (5/38)	0 (0/11)
++	0 (0/38)	0 (0/11)	0 (0/38)	0 (0/11)	0 (0/38)	0 (0/11)
++	0 (0/38)	0 (0/11)	0 (0/38)	0 (0/11)	0 (0/38)	0 (0/11)
H pylori infection					*P*<0.01	
Positive	28.9 (11/38)	54.5 (6/11)	31.5 (12/38)	18.2 (2/11)	36.9 (14/38)	9.0 (1/11)
BR+			36.9 (14/38)			
BR–			54.5 (6/11)			

more severe mucosal lesions, suggesting that bile reflux deteriorates mucosal lesions in the whole stomach. The exact mechanisms by which bile as well as other refluxing contents of duodenum cause gastric mucosal damage are still unclear. It has been indicated that interaction of bile acid, a component of bile, with M3 muscarinic receptor subtype expressed in chief cells may contribute to mucosal damage, manifested as active inflammation, intestinal metaplasia, glandular atrophy and focal hyperplasia, and other pathophysiological consequences of bile reflux[1,7,8-10].

Regurgitating via pylorus to gastric or esophageal cavity and sticking to mucous surface, refluxed bile might result in direct injuries of mucous epithelial cells and the tight junctions between these cells. It could provoke inflammatory responses in the whole mucous layer and submucous layer. Furthermore, by changing the chemical environment of mucous surface, regurgitated bile might affect the pathogenic patterns of other damage-causing factors such as gastric acid and *H pylori*, and potentiate the actions of these factors[1,2]. Consequently, bile reflux exerts harmful effects on gastric mucosa in the whole stomach.

Previous studies have shown that bile reflux is associated with the near-end gastric mucosal damage (including cardia cancer and intestinal metaplasia of cardia mucosa) as well as the mucosal lesions in bile reflux esophagitis[9,10]. Also, in the present study, the severity of mucosal pathological changes in the near-end stomach and *H pylori* infection in the corpus in bile reflux positive patients was significantly greater than that in patients without bile reflux. In addition, an obviously positive correlation was found between bile reflux time and gastric mucosal lesions including *H pylori* colonization in the near-end stomach, especially in the corpus, suggesting that bile reflux deteriorates gastric mucosal damage in the corpus region to a more extent, and bile reflux may facilitate *H pylori* colonization in gastric corpus. This phenomenon involves complex mechanisms. On the one hand, as described above, adhering to the surface of gastric corpus mucosa, refluxed bile might impair directly and strengthen the deleterious effects of gastric acid and pepsin on gastric corpus mucosa. On the other hand, as a result of adherence and destructive effects of regurgitated bile, the secretory and brushing capacity of gastric corpus mucosa was obviously decreased, which might facilitate *H pylori* colonization from the antrum to the corpus region and hence exacerbating gastric mucosal lesions in the corpus. The overall *H pylori* infection rate of bile reflux positive patients was lower than that of patients without bile reflux, but with no statistical significance. However, it was coincident with previous studies[1,2].

Combined with the pH monitoring system, the ambulatory Bilitec 2000 fiberoptic system could provide good approaches for simultaneous investigation of the dynamic processes of bile reflux and pH in stomach. The study of Barrett *et al*[5] showed that the method using Bilitec 2000 system for monitoring of bile reflux had a high sensitivity *in vivo*, while its sensitivity was much lower *in*

vitro, with a false-negative rate of 23%. In the acidic environment of the stomach, the values of bilirubin absorbance detected with this system were lower than their true values; the likelihood of false-positive results was very small. Consequently, it has been recognized that, although it may not be sufficiently reliable to be regarded as the gold standard for the evaluation of gastroduodenal reflux, this system is currently the most reliable method for dynamical monitoring of bile reflux, the results obtained can reflect the actual status of human body. The overall accuracy of this system should be sufficient for clinical use[5,11-15].

Theoretically, the existence of bile reflux in stomachs may at least, to some extent, reflect alkaline regurgitation from duodenum, which may result in decreased gastric H+ concentration. However, in our study, no obvious correlation was found between time of bile reflux and pH >4 in stomachs of dyspepsia patients. This was further confirmed in the real-time overlapped charts of bile absorbance and pH values. These findings may be due to the complex feedback regulatory networks between gastric acid secretory, gastric motion and emptying functions. The exact reasons why bile reflux failed to influence the acidic environment of gastric mucosa in dyspepsia patients remain to be determined.

In conclusion, the results of our study indicate that bile reflux affects both the anatomical and histological features of gastric mucosal damage and *H pylori* colonization in bile reflux positive patients. It may exacerbate near-end gastric mucosal damage, and facilitate *H pylori* colonization in the corpus region. Besides, bile reflux has no obvious influences on acid-exposing status of gastric mucosa in dyspepsia patients.

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