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GASTRITIS, GASTRIC ULCER, GASTRIC METAPLASIA: CLINICAL AND EXPERIMENTAL STUDIES

Investigation of the extent of gastric metaplasia in the duodenal bulb by using methylene blue staining

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Abstract

Background and Aims: The existence of gastric metaplasia (GM) of the duodenal mucosa has been considered to be highly related to the recurrence of duodenal ulcers (DU). The aims of this study are to evaluate the usefulness of methylene blue staining in the detection of GM, and to clarify the relationship between GM and the deformity of the duodenal bulb.

Methods: Fifteen patients with healed DU and four patients with symptoms of dyspepsia without evidence of ulcers were enrolled into this endoscopic study. During each endoscopy, methylene blue was sprayed evenly on the duodenal bulb, and biopsies were taken from blue-stained and unstained areas. The existence and extent of GM were assessed histologically and grossly. The correlation between duodenal bulb deformity and the extent of GM was also studied.

Results: The mean score of methylene blue non-staining (MBNS) was 0, 1.30 ± 0.15, and 3.00 ± 0.00 in group A (non-ulcer patients), group B (patients with healed DU and with normal-shaped bulb) and C (patients with healed DU and with deformed duodenal bulb), respectively; showing significant differences among the groups ($P < 0.05$ in each). Both the existence and the grading of GM were higher in unstained specimens than in blue-stained specimens (100 *vs* 16.6%, $P < 0.0001$ and 3.62 ± 0.09 *vs* 0.19 ± 0.06, $P < 0.001$, respectively).

Conclusions: Methylene blue non-staining can be applied to investigate the existence and extent of GM in the duodenal bulb accurately. The incidence of GM in the duodenal bulb was higher in patients with healed ulcers than in non-ulcer patients. Patients with deformed duodenal bulbs have a higher extent of GM than those without deformed duodenal bulbs.

Key words: duodenal ulcer, gastric metaplasia, methylene blue.

INTRODUCTION

The reported prevalence of gastric metaplasia (GM) of the mucosa of duodenal bulb in patients with duodenal ulcer (DU) ranges from 41 to 91%,[1–5] and it is significantly greater than that in non-ulcer controls.[6] Because GM of the duodenal mucosa has been considered to be an important factor for the development of DU,[2,3,5] identification of the extent of GM in the duodenal bulb may help us to understand the background of DU recurrence. In contrast, methylene blue can only be absorbed by matured intestinal epithelium, not by gastric epithelium. It can be used to identify the intestinal metaplasia in the stomach.[7–9] Recently, it was also applied to investigate the existence of GM in the duodenal bulb.[10] Our previous study revealed that DU patients with a marked deformed duodenal bulb had a higher incidence of GM than patients without bul-

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bar deformity.[11] To accurately identify GM further, we designed a prospective study to assess the accuracy of the methylene blue staining method in the detection of GM of the bulbar mucosa, and also to further study the relationship between GM and duodenal bulb deformity by using this dyeing method.

METHODS

This study was approved by the Human Subjects committee of our institute (Taipei Medical University Hospital). Nineteen patients including 13 men and six women, whose ages ranged from 31 to 66 years, were enrolled into the present study. Of the 19 patients, four were with symptoms of dyspepsia but without evidence of gastric or duodenal ulcer endoscopically, while 15 were with endoscopically proven healed DU that had been treated with an anti-*Helicobacter pylori* regimen (1 g amoxicillin, 500 mg clarithromycin and 20 mg omeprazole b.i.d. for 1 week) followed by 150 mg ranitidine b.i.d. for 4 weeks.

Upper gastrointestinal endoscopy was carried out with an Olympus GIF-XQ 240 endoscope (Olympus Optical Co. Ltd, Tokyo, Japan). Anisotropine methylbromide (Valpin, Sankyo Co. Ltd, Tokyo, Japan; 10 mg) was administered intramuscularly 5 min prior to endoscopic examination, and hyoscine butylbromide (Buscopan, Boehringer Ingelheim Pharma KG, Ingelheim am Rhein, Germany; 20 mg) was also given intravenously during this procedure if necessary to reduce duodenal peristalsis. During endoscopy, any abnormal finding of the duodenal bulb, including ulcer scars and deformity, was recorded before 10–20 mL 0.5% methylene blue was evenly sprayed into the duodenal bulb from every direction with a catheter. The methylene blue should have completely saturated the duodenal bulb and retained in the bulb for 5 min. Then, the duodenal bulb was carefully flushed with 20–40 mL distilled water, and the residual fluid and methylene blue was aspirated. We observed and recorded the area and extent of stained and unstained portions; two to three biopsy specimens were taken from the obviously stained and unstained areas with standard biopsy forceps (Olympus FB-25K-1; Olympus Optical Co. Ltd), but was not taken from the central part of ulcer scar if it was not stained. These specimens were fixed, embedded and then cut perpendicular to the mucosal surface; preparations, stained with hematoxylin and eosin and periodic acid–Schiff (PAS), were observed under a light microscope to identify the degree of gastric epithelialization, and to check whether there was existence of *H. pylori*. The histological assessment was performed by an expert pathologist who had no knowledge of the patient's clinical information.

Based on endoscopic morphological patterns of the duodenal bulb, duodenal ulcers were grouped into three types. Type I has a normal-shaped duodenal bulb, type II has a ridge across the bulb with pseudodiverticulum formation (mild degree of deformity), and type III has multiple ridges occupying the bulb (marked deformity).[11–13]

For comparing the existence and extent of the stained area of bulbar mucosa, we divided the patients into three groups. Group A consisted of non-ulcer patients, group B consisted of healed duodenal ulcer patients with normal-shaped bulbs (type I), and group C consisted of healed duodenal ulcer patients with deformity of the bulb (types II and III).

Scoring system for methylene blue non-staining

By visual assessment, we divided the extent of methylene blue non-staining (MBNS) of the duodenal bulb into four scores. Score 0 corresponded to no unstained area found in duodenal bulb (Fig. 1), score 1 corresponded to less than 25% non-stained surface of the duodenal bulb; score 2 corresponded to 25–50% non-stained surface (Fig. 2); and score 3 corresponded to more than 50% non-stained surface.

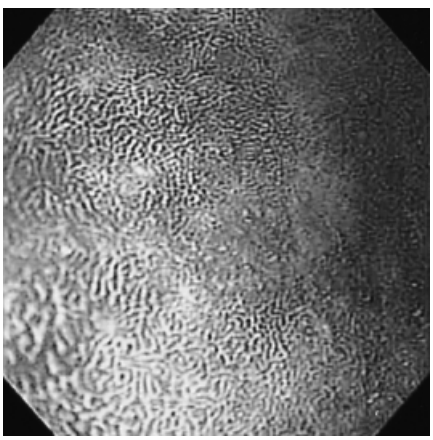

Figure 1 Score 0 of methylene blue non-staining: No unstained area was found in the duodenal bulb.

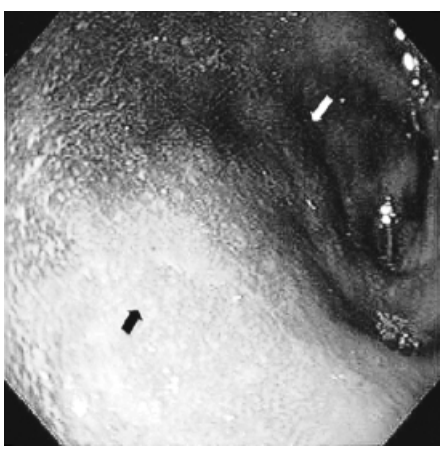

Figure 2 Score 2 of methylene blue non-staining. More than one-quarter of duodenal mucosa in the bulb was not stained by methylene blue (black arrow, unstained area; white arrow, stained area).

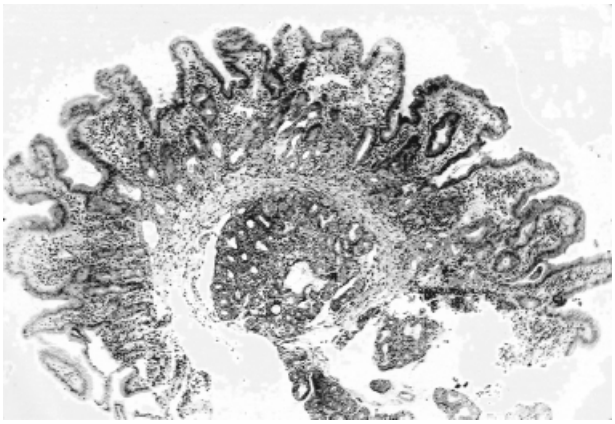

Figure 3 Grade 0 gastric metaplasia: normal appearance of the duodenal mucosa without evidence of gastric metaplasia (PAS stain ×100).

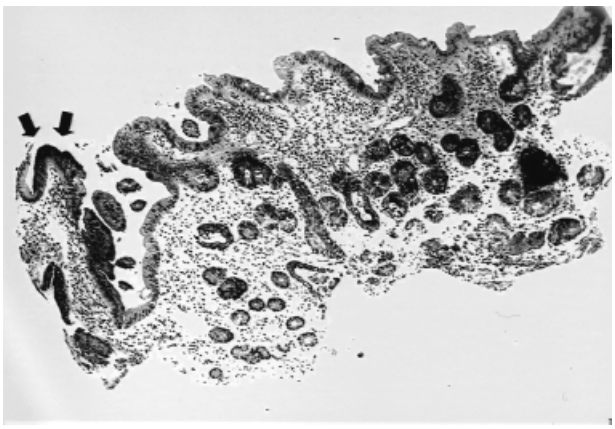

Figure 4 Grade 1 gastric metaplasia: less than 5% of duodenal mucosal cells contain PAS positive (arrows) gastric foveolar epithelium (PAS stain ×100).

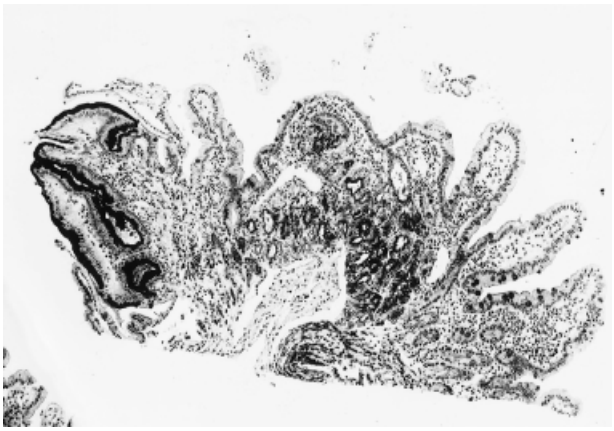

Figure 5 Grade 2 gastric metaplasia: less than one-quarter of duodenal mucosal cells have PAS-positive gastric-type epithelium (PAS stain ×100).

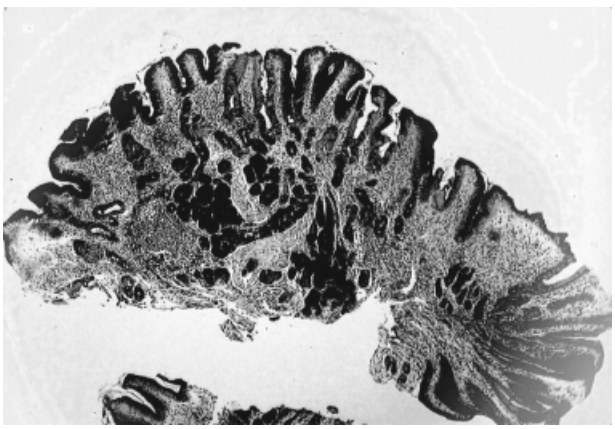

Figure 6 Grade 4 gastric metaplasia: more than 50% of mucosal surface were with gastric metaplasia (PAS stain ×100).

Grading system of gastric metaplasia microscopically

Histologically, we divided gastric metaplasia of the duodenal mucosa into five grades: (i) grade 0, no gastric-type epithelium found on duodenal mucosa (Fig. 3); (ii) grade 1, less than 5% duodenal mucosa contained gastric foveolar epithelium (Fig. 4); (iii) grade 2, 5–25% duodenal mucosa had gastric-type epithelium (Fig. 5); (iv) grade 3, 25–50% duodenal mucosal cells were with GM; (v) grade 4, more than 50% duodenal mucosa cells with GM (Fig. 6).

Statistical methods

The existence of GM in stained and unstained duodenal mucosa, and the incidences of GM in patients with and without duodenal ulcer were studied by using the chi-squared examination or Fisher's exact test. The grading of GM in stained and unstained duodenal mucosa and the extent (mean score) of MBNS in patients of groups A, B, and C were conducted by using a Student's *t*-test.

RESULTS

Of the 19 patients, four were in group A (without ulcer), 10 were in group B (healed ulcer with normal-shaped bulb) and five were in group C (healed ulcer with deformity of the bulb). The mean score (mean ± SD) of MBNS was 0, 1.30 ± 0.15, and 3.00 ± 0.00 in groups A, B and C, respectively; this showed statistically significant differences among the groups ($P < 0.05$ in each).

Microscopically, GM was found in the biopsy specimens of one (25%) of four cases of group A, and in all of 15 cases of groups B and C. It indicated that the incidence of GM in the duodenal bulb was 100% in patients with healed DU and 25% in non-ulcer patients. The difference between patients with and without DU was statistically significant ($P < 0.005$).

Table 1 Histological grading of gastric metaplasia (GM) of the biopsy specimens taken from stained and unstained areas of the duodenal bulb

Grade of GM	No. specimens	
	Unstained area	Blue-stained area
0 (0%)	0	40
1 (<5%)	0	7
2 (5–25%)	2	1
3 (25–50%)	7	0
4 (>50%)	28	0
Mean ± SD	3.62 ± 0.09*	0.19 ± 0.06*

*$P < 0.001$ by using a Student's *t*-test.

The numbers of duodenal biopsy specimens obtained from unstained areas and blue-stained areas were 37 and 48, respectively. Histologically, the existence of GM was 37 of 37 (100%) specimens in unstained areas, and eight of 48 (16.7%) specimens in blue-stained areas, showing statistically significant difference between them ($P < 0.0001$). The positive predictive value for GM in unstained duodenal mucosa was 100%. The negative predictive value for being free of GM in blue-stained duodenal mucosa was 83.3%.

As shown in Table 1, 35 of 37 duodenal biopsy specimens obtained from unstained areas were with high-grade GM histology (grades 3 or 4), and eight of 48 duodenal biopsy specimens obtained from blue-stained areas were with low-grade GM histology (grades 1 or 2). The difference of the grading of GM between stained and unstained duodenal mucosa was statistically significant (0.19 ± 0.06 *vs* 3.62 ± 0.09, $P < 0.001$). The methylene blue unstained duodenal mucosa showed 100% existence and a high-grade extent of GM.

Helicobacter pylori was not found in any biopsy specimen by the use of HE stain microscopically.

During and after this procedure, there were only minor side-effects to be noted in five patients. Four found blue-tinged stools and another found blue-colored urine for 1 day; no patients complained of obvious epigastric pain.

DISCUSSION

Chemical agents such as Lugol's solution, toludine blue, indigocarmine, Congo red and methylene blue have been applied to identify subtle carcinoma in gastrointestinal mucosa by using dyeing endoscopy.[8] Among them, methylene blue could be a good agent to detect intestinal-type gastric carcinoma, because it can be only absorbed by intestinal epithelium and not absorbed by gastric mucosa.[7–9] Methylene blue should be absorbed by the entire mucosa in the duodenal bulb, except the place where gastric-type epithelium exists. Gastric metaplasia has been considered as an important factor in the occurrence of DU, it is patchily distributed in the duodenal bulb. The existence and extent of GM was difficult to evaluate by the use of random biopsies. In the present study, we tried to identify GM in the duodenal bulb by negative staining of methylene blue. However, inadequate saturation of methylene blue in the duodenal bulb may affect the staining and result in false-positive predictions by visual assessment. Meanwhile, the occurrence of trauma in the duodenal mucosa when the duodenal epithelium is scraped by the catheter or scope may also disturb the absorption of methylene blue. Violently flushed distilled water into the duodenal bulb may also wash out the staining methylene blue. Hence, carefully performed endoscopy and spraying catheter are very important.

Our previous studies on the healing of DU have revealed that in the center of an ulcer scar might have poor maturity of the regenerating mucosa with lack of goblet cells.[14–16] Because the methylene blue dye is only absorbed (taken up) by the goblet cells, the central part of an ulcer scar may not be stained by methylene blue, owing to having a poorly matured regenerating mucosa instead of having GM; in the present study, we did not take biopsies from the center of the ulcer scar if it was not stained, to avoid the possible misinterpretation.

The pathogenesis of GM in the duodenal bulb is still unclear, but experimental evidence suggests a role for duodenal hyperacidity.[17] The occurrence of GM in the duodenal bulb may be a protective adaptation to acid. It has been reported that there is a higher incidence of GM in DU patients than in non-ulcer controls.[1,18] The present study also revealed that patients with a DU scar had a higher incidence of GM compared with non-ulcer patients.

In this series, a greater extent of GM (grades 3 or 4) was detected from the methylene blue-unstained areas of the duodenal bulb, and only low-grade GM (grades 1 or 2) could be detected from the methylene blue-stained area. The results are compatible with those of Howard *et al.*[10] The reason why we could detect GM (16.6%) in methylene blue-stained areas of the duodenal bulb was because more extensive blue-stained areas may obscure the limited unstained sites.

In our prior study, we found that a healed ulcer with a markedly deformed bulb had a higher incidence and extent of GM.[11] The similar result obtained was found to have a correlation existing between the extent of GM and deformity of the duodenal bulb. In the more deformed duodenal bulb, we could identify more unstained areas of methylene blue by visual assessment.

Random biopsies had been used to evaluate the relationship between GM and *H. pylori* infection in previous studies.[6,18,19] Because they did not detect the true area of GM prior to biopsy, it may lead to an over- or underestimation of the existence and extent of GM.

The present study demonstrated that endoscopic methylene blue staining could sensitively identify the existence of GM, and the grade of GM could be detected by following biopsies. Therefore, this methylene blue staining technique could be applied to accurately investigate the extent of GM in the duodenal bulb and may be very useful in the study of the relationship between GM and *H. pylori*.

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