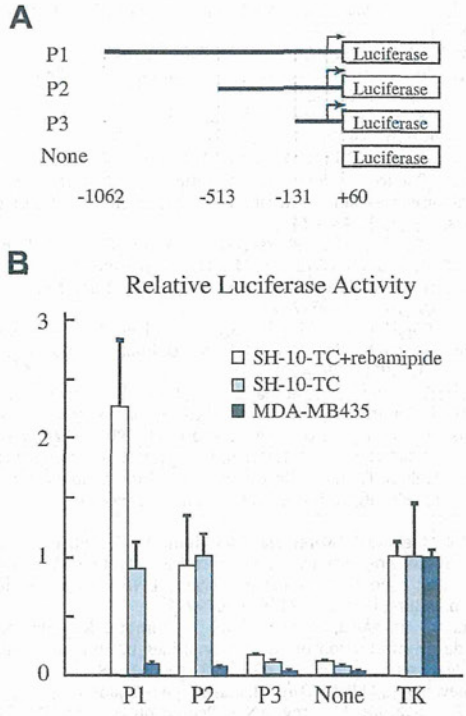

Fig. 4. (A) Schematic representation of the human *IL-1β* gene reporter constructs used in this study. The numbers represent base pairs measured from the 5′-end of the first exon of the *IL-1β* gene (+1), which is denoted as a rectangular arrow in each construct. (B) Transcriptional activities of a series of *IL-1β* promoter constructs in gastric SH-10-TC cells in the presence or absence of rebamipide. As a positive control, pGL4.12-TK (TK) harboring the human simplex virus thymidine kinase gene promoter was used. The promoter activities of MDA-MB435 cells deficient in *IL-1β* expression are also shown. Luciferase activities were measured 24 h after transfection, and the data shown are the mean values of triplicate experiments with the error bars corresponding to the standard errors.

cell growth [23,24]. These results suggest that rebamipide has some tumor-suppressive effects on gastric canceration.

In our present study, we discovered another possible anti-oncogenic effect of rebamipide upon gastric tumorigenesis, through the recruitment of dendritic cells capable of inducing tumor-specific immune responses [4]. There is a strong correlation between infiltration of dendritic cells to the tumor lesion and better prognosis in various cancers, including lung cancer [25], breast cancer [26], and hepatocellular carcinoma [27]. As for gastric cancer, there have also been several reports showing a strong correlation between the recruitment of dendritic cells and better prognosis [28–30]. In the MNNG-induced gastric cancer model rats treated with rebamipide, we detected a significant increase in dendritic cells in the precancerous gastric mucosa, which suggests a tumor-suppressive effect of the rebamipide administration.

We speculate that *IL-1β* transcriptional activation in gastric cells is one of the key mechanisms of the rebamipide-induced dendritic cell migration, but the precise mechanism of rebamipide on *IL-1β* gene promoter in gastric cells has not been elucidated. Contrary to our results, it was previously reported that rebamipide suppresses *IL-1β* expression in blood mononuclear cells [31]. The opposing results suggest that the transcriptional regulation of *IL-1β* gene in gastric epithelial cells is different from that of bone marrow-derived mononuclear cells. Considering the high-dose and long latent period required for the transcriptional activation of *IL-1β* gene by rebamipide, we presume that rebamipide-induced transactivation of *IL-1β* gene in gastric cells is due to an indirect effect of rebamipide via inducing some other transcription factors to the promoter region from −1062 to −513. Including validation of the possibility for direct binding of rebamipide to the *IL-1β* promoter region, the detailed mechanism of rebamipide on *IL-1β* gene transactivation needs to be resolved in the future. The variation of *IL-1β* gene regulation in different cell types is also a major problem that should be concurrently elucidated.

MNNG-treated rat stomachs and chronically *Helicobacter pylori*-infected human stomachs are quite different, but gastric mucosae in both cases are in a precancerous condition with chronic inflammation and upregulation of various cytokines [32]. We are planning to conduct a prospective cohort study of patients with chronic *Helicobacter pylori* infection, who will be treated with rebamipide or a placebo. Evaluation of dendritic cell migration in the presence or absence of rebamipide should be informative and suggestive for predicting its preventive effect on gastric tumorigenesis. Long-term follow-up focusing on gastric cancer incidence will finally provide clear evidence for our hypothesis of the tumor-suppressive effect of rebamipide.

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ORIGINAL ARTICLE

Relationship between vomiting reflex during esophagogastroduodenoscopy and dyspepsia symptoms

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Aim: Although frequent vomiting reflexes during esophagogastroduodenoscopy (EGD) causes suffering in patients, very few studies have investigated the characteristics of subjects who frequently develop vomiting reflexes. This study examined the incidence of the vomiting reflex and related factors, especially upper gastrointestinal symptoms, among individuals undergoing transoral EGD.

Methods: Subjects included 488 consecutive adults (mean age, 56.1 ± 8.9 years) who underwent transoral EGD for gastric cancer screening between February 2010 and March 2011. All procedures were performed by an endoscopist with 15 years of experience. Based on a questionnaire survey using the frequency scale for the symptoms of gastroesophageal reflux disease (FSSG), symptoms (dyspepsia and acid reflux symptoms) and the number of vomiting reflexes during EGD were recorded.

Results: Of the 488 subjects, 271 (56%) developed vomiting reflexes (mean, 4.2 times). This reflex-positive group was younger (54.3 ± 9.5 years) than the reflex-negative group (58.3 ± 7.7 years, $P < 0.001$). The number of subjects in the reflex-positive group with a high FSSG dyspepsia score (2.27 ± 2.57 *vs* 1.23 ± 1.84; $P < 0.001$), acid reflux symptom score (1.96 ± 2.22 *vs* 1.34 ± 2.14; $P < 0.01$) or an esophageal hiatal hernia (14.8% *vs* 4.6%; $P < 0.001$) was significantly higher than in the reflex-negative group. Multivariate analysis also showed a significant correlation between these four factors and the occurrence of vomiting reflexes. Using an FSSG dyspepsia score of 1 as the cut-off offered 68% sensitivity and 57% specificity for predicting the occurrence of vomiting reflexes.

Conclusion: Based on FSSG questionnaire responses on upper gastrointestinal symptoms, dyspepsia symptoms, in particular, are related to presence of vomiting reflexes during EGD.

Key words: conscious sedation, dyspepsia, gag reflex, transnasal endoscopy, vomiting reflex.

INTRODUCTION

Japan has a higher *Helicobacter pylori* infection rate than Western countries, and *Helicobacter pylori*-related chronic gastritis is a main background factor in many cases of gastric cancer. To reduce the related high mortality and morbidity rates, mass screening for gastric cancer has been conducted as a public health service since the mid-1960s.[1] Gastric cancer screening with esophagogastroduodenoscopy (EGD) has gradually gained popularity in Japan in place of conventional screening with barium X-ray.[2,3] However, EGD is an invasive procedure that entails considerable discomfort, so minimizing the distress of subjects who undergo gastric cancer screening with EGD is important.

The vomiting reflex that occurs frequently during EGD is one response encountered in patients who feel discomfort. Transnasal EGD is reportedly associated with fewer vomiting reflexes and less discomfort compared to conventional transoral EGD.[4–7] Therefore, transnasal EGD has increased in popularity recently in Japan, with the aim of alleviating discomfort. However, as the number of EGD for gastric cancer screening increases, problems are increasingly encountered regarding the manpower needed for the complex pretreatment for transnasal EGD and patient safety (i.e. adverse effects from sedatives). These issues make transnasal EGD and conscious sedation difficult to apply in all patients. In addition, many endoscopists have encountered a fair number of patients who show absolutely no vomiting reflex during transoral EGD. However, very few reports have examined which subjects are more likely to show a vomiting reflex, and the details of this reflex remain unclear.

Functional dyspepsia (FD) is a typical functional gastrointestinal disorder,[8] and it has been suggested that it occurs as a result of many factors,[9] including impaired gastric accommodation. Gastric accommodation is thought to reflect gastric retention function and to be related to dyspepsia symptoms such as the amount of food that can be ingested and sense of satiation.[10–13] In EGD, factors associated with the vomiting reflex are thought to be air insufflation and gastric

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irritation caused by the endoscope, which, in turn, cause the gastric walls to distend and stimulate the vomiting center via sympathetic and vagal afferents.[14] Impaired gastric accommodation in response to distention of the gastric wall by insufflation and the endoscope during the procedure may be one cause of the vomiting reflex.

The purpose of this study was to investigate the relationship between the presence or absence of the vomiting reflex and various background and clinical factors in subjects who underwent transoral EGD for gastric cancer screening. In particular, the relationship between upper gastrointestinal symptoms and the vomiting reflex were closely examined.

METHODS

Study population

In Japan, health checkups are performed to identify selected diseases (e.g. gastric cancer) in the early stage of development. Subjects included 488 consecutive adults (409 men, 79 women; mean age, 56.1 ± 8.9 years) who underwent transoral EGD for gastric cancer screening. A single endoscopist (SE; 15 years of experience, during which he performed transoral EGD in more than 5000 patients) performed all procedures at the Wakayama Wellness Foundation (Wakayama, Japan) between February 2010 and March 2011. The study protocol was approved by the ethics committee of Wakayama Medical University (Wakayama, Japan) and informed consent was obtained from all subjects.

Endoscopic procedure

EGD was performed in a standardized fashion for the main purpose of gastric cancer screening, but also for differential diagnosis of other esophageal, gastric and duodenal diseases. The endoscope was pushed to the esophagogastric junction via the mouth, and then through the stomach and pylorus into the descending duodenum. The endoscope was then pulled back into the stomach for inspection of the gastric antrum and corpus. Gastric angulation and gastric cardia were inspected in inversion. The endoscope was removed slowly, after careful inspection of the stomach and esophagus. Three endoscopes were used, including two GIF-XP240 (tip outer diameter, 7.7 mm) and one GIF-XQ230 (tip outer diameter, 9.2 mm) from Olympus Medical Systems (Tokyo, Japan).

For EGD preparations, 22.5 mL water was added to 2.5 mL 4% lidocaine hydrochloride solution (Xylocaine; AstraZeneca, Osaka, Japan) and then gargled to a total of 25 mL (10-fold dilution). Next, 100 mL water was added to 5 mL 2% dimethicone syrup (Gascon; Kissei Pharmaceutical, Nagano, Japan), and 1 g sodium bicarbonate (Sioe Pharmaceutical, Hyogo, Japan) and 0.5 g pronase (Pronase MS; Kaken Pharmaceutical, Tokyo, Japan) were dissolved and administered. Third, 8% lidocaine hydrochloride spray (AstraZeneca) was sprayed into the pharynx. Subjects who desired conscious sedation during the test were given intravenous diazepam (Cercine; Takeda Pharmaceutical, Osaka, Japan) at 5–7.5 mg/body (5 mg/body for patients ≥70 years old or with body weight ≤50 kg) immediately before EGD.

Assessment of upper gastrointestinal symptoms

A medical questionnaire was used for objective evaluation of upper gastrointestinal symptoms. Various medical questionnaires have been devised for gastroesophageal reflux disease (GERD).[15] In Japan, the frequency scale for the symptoms of GERD (FSSG) developed by Kusano *et al.* is often used.[16] The FSSG questionnaire uses 12 questions,scored to indicate the frequency of symptoms as follows: never = 0, occasionally = 1, sometimes = 2, often = 3, and always = 4. Of the 12 questions on the FSSG, seven relate to acid reflux symptoms and five to dyspepsia symptoms. Evaluations can be performed separately for these two symptom systems.[17–19] This makes the FSSG useful as a diagnostic tool not only for GERD, but also for FD. FSSG was used in this study, and subjects completed the questionnaire with regards to upper gastrointestinal symptoms encountered in the preceding 2–3 weeks. Evaluations were performed for acid reflux symptom score, dyspepsia symptom score, presence or absence of acid reflux symptoms, presence or absence of dyspepsia symptoms, and the 12 questions on the FSSG.

Parameters assessed

The number of vomiting reflexes by each patient during the procedure (from endoscope insertion into the pharynx until withdrawal) was recorded by the endoscopist or nursing staff. Anything thought to be vomiting, belching or retching was recorded in the number of positive reflexes. Obvious coughs were not recorded in the number of reflexes. Factors related to the presence or absence of the vomiting reflex were investigated. In addition to upper gastrointestinal symptoms assessed by the FSSG, parameters investigated were age, sex, body mass index, current drinking habits, current smoking habits, history of gastrectomy, administration of acid reducers (proton pump inhibitor or histamine-2 receptor antagonist), past experience of EGD, differences in endoscope used, whether the subjects desired conscious sedation during the procedure, time for EGD, presence or absence of reflux esophagitis (Los Angeles classification system:[20] Grades A–D indicate erosive esophagitis), and presence or absence of hiatal hernia (hiatal hernia was diagnosed endoscopically when the distance between the crural impression and gastroesophageal junction was ≥2 cm).

Statistical analysis

Univariate analysis was performed using the unpaired *t*-test for numerical data and Fisher's exact test for categorical data. Multivariate analysis was performed using a logistic regression model. All tests were two-sided, and values of $P < 0.05$ were considered statistically significant. All analyses were performed using SPSS software (SPSS, Chicago, IL, USA).

RESULTS

Of the 488 subjects, 271 (56%) experienced ≥1 vomiting reflex during EGD. The mean number of vomiting reflexes in these subjects was 4.2. Subjects with ≥1 vomiting reflex were placed in a reflex-positive group, and those without vomiting reflex in a reflex-negative group. Various factors were

compared between reflex-negative and reflex-positive groups (Tables 1, 2). Age was significantly lower in the reflex-positive group ($P < 0.001$). The reflex-positive group also had significantly higher FSSG scores for acid reflux symptoms ($P < 0.01$) and dyspepsia symptoms ($P < 0.001$), and significantly higher rates of acid reflux symptoms ($P < 0.001$) and dyspepsia symptoms ($P < 0.001$). Findings of hiatal hernia were present at a higher rate in the reflex-positive group ($P < 0.001$). The percentage of cases in which conscious sedation was performed according to the wishes of the patient was significantly higher in the reflex-positive group ($P < 0.001$). Since effects of conscious sedation on vomiting reflex could not be ignored in this study, a separate investigation of subjects without conscious sedation (reflex-negative, $n = 125$; reflex-positive, $n = 106$) and with conscious sedation (reflex-negative, $n = 92$; reflex-positive, $n = 165$) was performed (Table 1,2). The results of analysis of these subgroups did not differ markedly from the results of the overall analysis. Age was significantly lower, FSSG acid reflux symptom and dyspepsia symptom scores were significantly higher, and the rate of findings of hiatal hernia was significantly higher in the reflex-positive group than in the reflex-negative group for subjects both with and without conscious sedation. However, findings of erosive esophagitis were significantly higher in the reflex-positive group than in the reflex-negative group among subjects with conscious sedation ($P < 0.05$), whereas no significant difference was apparent among subjects without conscious sedation.

A comparison of each of the 12 FSSG questions between groups showed that scores for all five questions related to dyspepsia symptoms (Questions 2, 3, 5, 8, and 11) were higher in the reflex-positive group than in the reflex-negative group (Table 3).

Multivariate analysis was performed for vomiting reflex using the four factors of age, presence or absence of hiatal hernia, acid reflux symptoms and dyspepsia symptoms. Age was a significant negative independent factor for vomiting reflex ($P < 0.001$), and hiatal hernia ($P < 0.001$), acid reflux symptoms ($P < 0.05$) and dyspepsia symptoms ($P < 0.01$) were significant positive independent factors (Table 4).

Using a cutoff value of 1 for the FSSG dyspepsia symptom score achieved 68% sensitivity and 57% specificity with respect to predicting presence of the vomiting reflex.

DISCUSSION

In this study, the vomiting reflex was seen in the majority of subjects who underwent gastric cancer screening using transoral EGD. Although conscious sedation with diazepam or midazolam is used to overcome the vomiting reflex, these medications are sometimes not helpful and most sedated subjects are still able to vomit.[21] Similarly, the vomiting reflex-positive group in the present study included a larger number of subjects with conscious sedation using diazepam. Many of the present subjects had undergone EGD in the past and may have chosen conscious sedation because of the discomfort they had experienced from the vomiting reflex at that time. During endoscopy and fine endoscopic examinations, procedures take a substantial amount of time, and sedation is necessary. However, in the present study, the average time for transoral EGD examination for the purpose of

Table 1. Comparison of subjects' background factors according to presence of vomiting reflex

	Total subjects		*P*-value	Subjects without conscious sedation		*P*-value	Subjects with conscious sedation		*P*-value
	Reflex-negative ($n = 217$)	Reflex-positive ($n = 271$)		Reflex-negative ($n = 125$)	Reflex-positive ($n = 106$)		Reflex-negative ($n = 92$)	Reflex-positive ($n = 165$)	
Age (years, mean ± SD)	58.30 ± 7.66	54.32 ± 9.45	<0.001	59.12 ± 7.56	56.00 ± 9.94	0.009	57.17 ± 7.70	53.24 ± 8.98	<0.001
Sex (men/women)	185/32	224/47	0.460	106/19	88/18	0.722	79/13	136/29	0.598
Body mass index (mean ± SD)	23.38 ± 3.12	23.66 ± 3.19	0.326	23.60 ± 3.06	23.59 ± 3.03	0.991	23.09 ± 3.20	23.71 ± 3.29	0.144
Current drinking (−/+)	73/144	100/171	0.505	44/81	41/65	0.588	29/63	59/106	0.584
Current smoking (−/+)	155/62	177/94	0.172	94/31	82/24	0.758	61/31	95/70	0.185
Past history of gastrectomy (−/+)	214/3	267/4	1.000	124/1	104/2	0.594	90/2	163/2	0.619
Medication of PPI or H2RA (−/+)	214/3	265/6	0.737	123/2	104/2	1.000	91/1	161/4	0.657

H2RA, histamine-2 receptor antagonist; PPI, proton pump inhibitor.

Table 2. Comparison of subjects' clinical factors according to presence of vomiting reflex

	Total subjects		*P*-value	Subjects without conscious sedation		*P*-value	Subjects with conscious sedation		*P*-value
	Reflex-negative ($n = 217$)	Reflex-positive ($n = 271$)		Reflex-negative ($n = 125$)	Reflex-positive ($n = 106$)		Reflex-negative ($n = 92$)	Reflex-positive ($n = 165$)	
FSSG									
Acid reflux symptom score (mean ± SD)	1.34 ± 2.14	1.96 ± 2.22	0.002	1.56 ± 2.18	2.19 ± 2.29	0.034	1.03 ± 2.06	1.82 ± 2.17	0.005
Acid reflux symptoms (−/+)	118/99	89/182	<0.001	61/64	30/76	0.002	57/35	59/106	<0.001
Dyspepsia symptom score (mean ± SD)	1.23 ± 1.84	2.27 ± 2.57	<0.001	1.30 ± 1.76	2.14 ± 2.34	0.003	1.13 ± 1.94	2.35 ± 2.71	<0.001
Dyspepsia symptoms (−/+)	123/94	87/184	<0.001	66/59	37/69	0.007	57/35	50/115	<0.001
EGD									
Past experience of EGD (−/+)	14/203	14/257	0.562	8/117	7/99	1.000	6/86	7/158	0.553
Endoscope (GIF-XP240 [7.7 mm]/GIF-XQ230 [9.2 mm])	182/35	222/49	0.630	102/23	87/19	1.000	80/12	135/30	0.379
Conscious sedation (−/+)	125/92	106/165	<0.001	–	–	–	–	–	–
Endoscopic biopsy (−/+)	201/16	259/12	0.175	115/10	99/7	0.803	86/6	160/5	0.208
Time for EGD (s: mean ± SD)	424.98 ± 21.80	422.88 ± 14.78	0.226	425.28 ± 24.12	424.53 ± 19.77	0.798	424.57 ± 18.30	421.81 ± 10.32	0.187
Erosive esophagitis (−/+)	182/35	211/60	0.107	101/24	84/22	0.869	81/11	127/38	0.032
Hiatal hernia (−/+)	207/10	231/40	<0.001	119/6	92/14	0.033	88/4	139/26	0.007

EGD, esophagogastroduodenoscopy; FSSG, frequency scale for the symptoms of gastroesophageal reflux disease.

gastric cancer screening was approximately 8 min, which is relatively not long. In addition, close to half of the subjects did not have a vomiting reflex, and given the poor effect of diazepam in reducing the vomiting reflex and problems such as the occurrence of accidental symptoms with sedation, the need for conscious sedation in all patients remains highly debatable.

In this study, many subjects with dyspepsia symptoms were in the reflex-positive group, suggesting that impaired gastric accommodation may be a factor in the vomiting reflex. However, dyspepsia symptoms are not only seen in FD patients. The results of this study showed no relationship between the vomiting reflex and reflux esophagitis. Moreover, while a stronger relationship was seen with dyspepsia symptoms than with acid reflux symptoms, the finding that younger people and hiatal hernias were more common in the reflex-positive group also suggests a relationship with GERD. However, the fact that no previous reports have described a high frequency of impaired gastric accommodation or FD among younger people is inconsistent with the finding of many young people being reflex-positive in the present study. This suggests that the vomiting reflex is influenced by multiple factors. Accordingly, there is a high likelihood that FD and GERD are complicating conditions, and in fact, physiological assessment using tests,[22] such as fundic barostat, single photon emission computed tomography, or ultrasonography, is probably necessary to demonstrate a relationship between impaired gastric accommodation and the vomiting reflex.

The vomiting reflex during EGD is not thought to have a single trigger. The vomiting reflex is also called the gag reflex or pharyngeal reflex, and may even be produced by touching the pharynx (especially the base of the tongue) with an endoscope. As the transnasal endoscope is inserted without touching the tongue and does not induce the reflex, several studies have reported this approach as being significantly more patient-friendly than conventional transoral endoscopy.[4–7] Contrary to the results of those investigations, other studies have reported no significant differences in the number of occurrences of vomiting reflex between transnasal and transoral groups.[23–25] Given that no vomiting reflex was seen in about half of subjects who underwent transoral EGD in our study, contact stimulus at the base of the tongue alone is not likely induce vomiting in all cases.

The mean age of subjects in this study was relatively low. Age was a negative independent factor for the vomiting reflex, but the percentage of subjects showing a vomiting reflex may differ greatly depending on the age makeup of the subject cohort. Subjects in this study were people who underwent gastric cancer screening, typically because of suspicious findings from the FSSG questions. Generally, people who undergo gastric cancer screening do not have strong subjective symptoms that prompt a hospital visit for an examination. In studies like this one that look at people who undergo EGD for both general screening and for close investigation of existing symptoms, the percentage of people with a vomiting reflex may be increased.

The present study had several limitations that must be considered when interpreting the results. First, the investigation was based on results from EGD performed by a single endoscopist. However, if the procedure had been performed by multiple endoscopists, varying EGD skill level resulting

Table 3. Comparison of FSSG score according to presence of vomiting reflex

FSSG question			Vomiting reflex-negative group ($n=217$)	Vomiting reflex-positive group ($n=271$)	P-value
Q1	A	Do you get heartburn?	0.295 ± 0.598	0.428 ± 0.679	0.022
Q2	D	Does your stomach get bloated?	0.309 ± 0.602	0.542 ± 0.773	<0.001
Q3	D	Does your stomach ever feel heavy after meals?	0.286 ± 0.586	0.564 ± 1.030	<0.001
Q4	A	Do you sometimes subconsciously rub your chest with your hand?	0.111 ± 0.405	0.170 ± 0.480	0.140
Q5	D	Do you ever feel sick after meals?	0.065 ± 0.281	0.182 ± 0.488	0.001
Q6	A	Do you get heartburn after meals?	0.217 ± 0.475	0.373 ± 0.588	0.001
Q7	A	Do you have a burning sensation in your throat?	0.194 ± 0.561	0.277 ± 0.684	0.140
Q8	D	Do you feel full while eating meals?	0.180 ± 0.518	0.284 ± 0.612	0.042
Q9	A	Do some things get stuck when you swallow?	0.148 ± 0.437	0.214 ± 0.529	0.129
Q10	A	Do you get bitter liquid coming up into your throat?	0.346 ± 0.613	0.483 ± 0.660	0.017
Q11	D	Do you burp a lot?	0.364 ± 0.740	0.657 ± 0.863	<0.001
Q12	A	Do you get heartburn if you bend over?	0.051 ± 0.259	0.207 ± 1.856	0.174

A, acid reflux symptom; D, dyspepsia symptom; FSSG, frequency scale for the symptoms of gastroesophageal reflux disease.

Table 4. Factors associated with vomiting reflex: results of multivariate logistic analysis

Factor	P-value	Odds ratio	95%CI
Age (years)	0.001	0.96	0.93–0.98
Hiatal hernia (–/+)	0.001	3.5	1.67–7.35
Acid reflux symptoms (–/+)	0.046	1.56	1.01–2.39
Dyspepsia symptoms (–/+)	0.002	1.99	1.29–3.06

CI, confidence interval.

from differences in the number of years of experience or other factors would be unavoidable. Investigations performed by a single endoscopist have the advantage of eliminating this point.

Second, no special instruments were used for measurements in recording the number of vomiting reflexes, and objectivity may thus have suffered. The vomiting reflex has a variety of causes, including insufflation during the procedure, gastric distention by the endoscope and stimulation of the base of the tongue; it cannot be clearly distinguished which one produces the reflex in each case. Similarly, vomiting, belching, retching or just excretions of air are totally different phenomena, and fundamentally distinguishing between them would be useful in pathological analysis of the vomiting reflex. However, in practical terms, making these distinctions is quite difficult, and attempting to do so may have the effect of decreasing accuracy. Therefore, anything thought to represent one of these symptoms as a positive reflex was recorded. However, the vomiting reflex was compared between a reflex-negative group, in which no vomiting reflexes were encountered, and a reflex-positive group showing ≥1 vomiting reflexes. An assessment of no vomiting reflexes at all is thought to offer a relatively high degree of objectivity, and in an investigation dividing patients into two groups such, this is not considered a particularly large problem.

A third limitation was that the relationship between endoscopic manipulation and the vomiting reflex could not be analyzed. Thus, the records do not clearly distinguish between vomiting reflex due to air insufflation of the stomach and vomiting reflex due to pharyngeal stimulation on insertion of the endoscope. Endoscopic manipulations such as insufflation or insertion of the endoscope represent coordinated manipulations, and since they are not always performed independently, discriminating between these two operations is likely to prove difficult. However, if the relationship between endoscopic manipulation and the vomiting reflex could be recorded and analyzed, more accurate evaluation of the cause of the reflexes would be possible. Studies from this perspective will be necessary in the future.

For the vomiting reflex, mainly background factors were investigated, but no objective evaluation of distress in subjects was performed. For example, no evaluation was undertaken of whether there was truly little distress in the vomiting reflex-negative group or whether little distress was seen as a result of conscious sedation even in the reflex-positive group. In the future, evaluation of distress using a visual analog scale or other tools will be necessary.[26]

In conclusion, this study revealed a relationship between the vomiting reflex, dyspepsia and other upper gastrointestinal symptoms. Symptoms can be objectively assessed with the use of medical questionnaires such as the FSSG, and prediction of the presence or absence of the vomiting reflex during EGD may be feasible. Relationships were suggested between the vomiting reflex and FD or GERD. With conventional transoral EGD, a majority of subjects showed a vomiting reflex, but some showed no reflex at all. Further investigation is warranted regarding the kinds of subjects in whom conscious sedation or transnasal EGD are appropriate.

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LETTERS, TECHNIQUES AND IMAGES

Phlegmonous gastritis caused by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA)

Phlegmonous gastritis (PG) is a rare, often fatal, condition characterized by suppurative bacterial infection of the stomach.[1] Mucosal damage of the stomach, alcoholism and an immunocompromised state are predisposing factors.[2] Phlegmonous gastritis rarely develops after therapeutic endoscopy and only a few instances have been reported.[3] We describe a patient with PG that arose as a complication after endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA).

A 70-year-old woman with a diagnosis of pancreatic tumor attended our hospital. A 50-mm, low echoic lesion at the pancreatic body was identified by EUS and EUS-FNA proceeded through the stomach using a 19-gauge needle (Echo Tip®Ultra; Wilson-Cook, Winston Salem, NC, USA). She was discharged on the following day. She returned to our hospital 1 week later due to persistent upper abdominal pain and low-grade fever. Her vital signs were: blood pressure, 105/66 mmHg; regular pulse, 96 b.p.m. and temperature of 38°C.

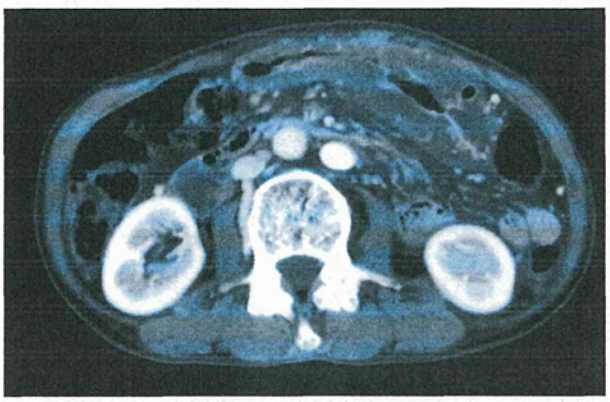

Fig. 1. Multi-detector computed tomography image. Air is trapped in diffusely thickened gastric wall.

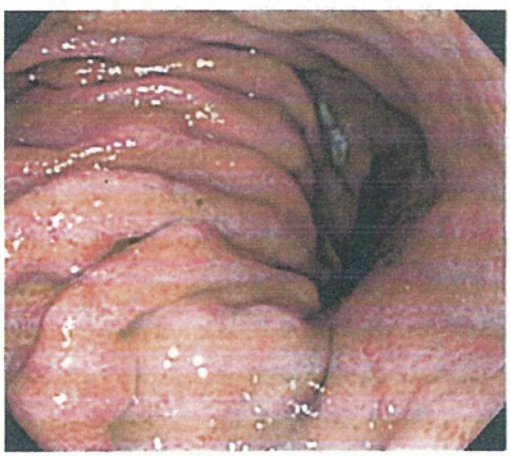

Fig. 2. Upper gastrointestinal endoscopy shows diffuse erythema, edema and erosions.

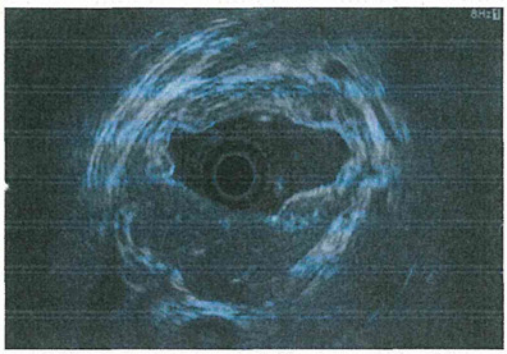

Fig. 3. Endoscopic ultrasound shows submucosa of gastric wall is predominantly thickened.

The patient's abdomen was soft, flat, and slightly distended with mild tenderness in the upper area. The laboratory findings were as follows: obvious inflammation with white blood cells (WBC) 13 500/μL and C-reactive protein (CRP) 28 mg/dL. Multi-detector row computed tomography (MDCT) revealed diffuse thickening of the gastric wall and air trapped within it (Fig. 1). Upper gastrointestinal endoscopy revealed diffuse erythema, edema and erosions (Fig. 2). EUS also showed diffuse gastric wall thickening, predominantly in the submucosa (Fig. 3). The culture of several biopsy specimens from the mucosal surface revealed *α-Streptococcus* and PG caused by EUS-FNA was clinically diagnosed. The patient recovered after antibiotic therapy with peperacillin/tazobactam and she was discharged on hospital day 15.

EUS-FNA is a safe procedure with a complication rate of approximately 1% that does not normally require antibiotic prophylaxis.[4] However, the risk of PG must be considered for immunocompromised patients with advanced cancer and preventative antibiotics may be necessary.

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ORIGINAL ARTICLE

Diazepam during endoscopic submucosal dissection of gastric epithelial neoplasias

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Abstract

AIM: To investigate risk factors and adverse events related to high-dose diazepam administration during endoscopic submucosal dissection for gastric neoplasias.

METHODS: Between February 2002 and December 2009, a total of 286 patients with gastric epithelial neoplasia underwent endoscopic submucosal dissection in our hospital. To achieve moderate sedation, 5-7.5 mg of diazepam was administered intravenously by non-anesthesiologists. Intermittent additional administration of 2.5-5 mg diazepam was performed if uncontrollable body movement of the patient was observed. All patients were classified into groups based on the required diazepam dose: low-dose ($\leqslant$ 17.5 mg, n = 252) and high-dose (> 17.5 mg, n = 79).

RESULTS: Differences between the low- and high-dose diazepam groups were observed in lifetime alcohol consumption (0.30 ± 0.48 *vs* 0.44 ± 0.52 tons, P = 0.032), body weight (58.4 ± 10.3 *vs* 62.0 ± 9.9 kg, P = 0.006), tumor size (15 ± 10 *vs* 23 ± 18 mm, P < 0.001), lesion location (P < 0.001) and the presence of ulcerative findings (14/238 *vs* 18/61, P < 0.001). Multivariate analysis identified all five variables as independently related to required diazepam dosage. In terms of adverse reactions to diazepam administration, paradoxical excitement was significantly more frequent in the high-dose diazepam group (P < 0.001).

CONCLUSION: Intermittent administration of diazepam enabled safe completion of gastric endoscopic submucosal dissection except in patients who were alcohol abusers or obese, or who showed complicated lesions.

Key words: Diazepam; Endoscopic submucosal dissection; Gastric epithelial neoplasias; Moderate sedation; Non-anesthesiologists

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INTRODUCTION

Endoscopic submucosal dissection (ESD) is a novel and minimally invasive procedure for the treatment of gastric epithelial neoplasia. As this technique permits en bloc resection of lesions, ESD has the advantages of enabling accurate pathological assessment and reducing the risk of local recurrence[1]. However, in comparison to conventional endoscopic mucosal resection (EMR), ESD requires a high level of endoscopic competence and a longer resection time[2-4]. In addition, many cases of early gastric cancer occur in elderly patients, who also display increased sensitivity to sedatives and a higher risk of adverse reactions, including respiratory and cardiovascular depression[5]. Suitable sedatives that do not cause complications and permit safe completion of ESD thus need to be identified.

The American Society of Anesthesiologists (ASA) classifies the degree of sedation into four levels: minimal sedation; moderate or conscious sedation; deep sedation; and general anesthesia[6]. Given that deep sedation or even general anesthesia can be achieved with propofol, the ASA suggests that care must be taken even if aiming for moderate sedation[6]. In addition, due to the narrow therapeutic window[7-9], the American Society for Gastrointestinal Endoscopy has recommended the presence of trained personnel dedicated to the administration of propofol[10]. To date, the safety and efficacy of sedation using propofol have been reported in esophagogastroduodenoscopy, colonoscopy, endoscopic ultrasonography and endoscopic retrograde cholangiopancreatography[11-15]. In contrast, due to the risk of cardiorespiratory complications, particularly in the elderly, the Japan Gastroenterological Endoscopy Society does not recommend sedation using propofol for endoscopic procedures. Thus, there is an in-principle requirement in Japan that propofol be administered by an anesthesiologist. As a result, not many institutions use propofol for sedation during ESD[16,17].

Of the available sedatives, benzodiazepines are generally considered to have a broad safety margin as they do not activate the gamma-aminobutyric acid $(GABA)_A$ receptor in the absence of endogenous GABA[18]. Diazepam is the least potent injectable benzodiazepine sedative, with a long history of clinical use, even by non-anesthesiologists. Moreover, unlike in the case of propofol administration, if a patient falls into deep sedation while being treated with diazepam, a pharmacological antagonist (flumazenil) can be administered to counter this effect[19,20]. Fujishiro *et al*[21] reported that, in principle, ESD for esophageal squamous cell neoplasms could be performed with the patient under conscious sedation induced by intermittent administration of diazepam and pentazocine. However, administration methods have yet to be clearly established for safe and effective sedative use during the gastric ESD procedure.

The objectives in this retrospective study were to evaluate variables relating to the diazepam dosage during ESD for gastric epithelial neoplasia and to investigate the characteristics and adverse events of patients administered high-dose diazepam.

MATERIALS AND METHODS

Patients

Between February 2002 and December 2009, we performed ESD for 446 gastric epithelial neoplastic lesions in 342 consecutive patients treated at Wakayama Medical University Hospital. ESD was indicated for patients with adenomas suspected of being malignant on the basis of endoscopic findings or biopsy. In addition, ESD was indicated for patients with early gastric cancers that were considered to have a nominal risk of lymph node metastasis according to the criteria of Gotoda *et al*[22], excluding undifferentiated cancers. For this study, we retrospectively analyzed ESDs that had been performed for 331 lesions in 286 patients (mean age, 69.5 years; range, 42-90 years). Excluded lesions comprised 77 cases for which multiple lesions had been simultaneously dissected by ESD, 26 cases for which diazepam had not been administered, 7 lesions in which other investigations had been carried out, and 7 lesions for which the intraoperative records were unclear (with an overlap of 2 lesions). All patients underwent blood tests, chest X-rays and electrocardiographic testing before treatment. ESD was indicated for patients with an ASA classification of 1-3[23]. This study was approved by the ethics committee of Wakayama Medical University, and all patients provided written informed consent prior to undergoing ESD.

ESD procedures

ESD was performed by one of four experienced therapeutic endoscopists, each of whom had performed ESD for more than 50 cases of early gastric cancer or gastric adenoma. We predominantly used a flex electrosurgical knife (KD-630L; Olympus, Tokyo, Japan)[2,24], along with a hook knife (KD-620LR; Olympus) when necessary[25]. Hemostatic forceps (HDB2422W; Pentax, Tokyo, Japan)[26-28] were used to reduce bleeding during ESD.

Diazepam administration

We aimed to achieve moderate sedation during ESD. For introduction, we intravenously administered diazepam (Cercine®; Takeda Pharmaceutical, Osaka, Japan) at 5-7.5 mg/body (5 mg/body for patients $\geqslant$ 75 years old or weighing $\leqslant$ 50 kg) prior to insertion of the endoscope; in principle, administration of diazepam was continued

up to 10 mg during ESD. When the sedative effect of 10 mg diazepam was judged sufficient, administration of the drug was continued without any change, and additional administration was performed in intermittent doses of 2.5-5 mg/body each, only when uncontrollable body movement was observed (maximal dose: 40 mg). When the sedative effect of 10 mg diazepam was judged to be insufficient and patient distress was considered great, diazepam was switched to midazolam (Dormicum®; Astellas Pharmaceutical, Tokyo, Japan) for rescue, administered intermittently at 1-2 mg/body. Intermittent sedative administration was performed by non-anesthesiologists (i.e., gastroenterologists) at the direction of the operator. For the purposes of pain relief, 15 mg of pentazocine (Sosegon®; Astellas Pharmaceutical) was administered intramuscularly to all patients at the start of ESD. When the level of anesthesia reached deep sedation, flumazenil (Anexate®; Astellas Pharmaceutical) was administered as deemed necessary.

Patient monitoring

Blood pressure, heart rate, electrocardiography (ECG), and peripheral oxygen saturation (SpO_2) were monitored during the procedure. Blood pressure was measured at 5-min intervals, while heart rate, ECG tracing and SpO_2 were measured continuously. Supplementary oxygen was administered to patients with SpO_2 below 90%. Administered dosages of sedatives and analgesics, all adverse events (such as decreases in SpO_2 below 90% and blood pressure below 90 mmHg), and uncontrollable body movements were recorded by trained nurses.

Patients were instructed to rest in bed for 3 h following ESD, and to remain under strict observation until the next morning. All ESD procedures were performed on an inpatient basis, and patients were discharged within 10 days after ESD if no problems were encountered.

Parameters assessed

Since several reports have indicated that it is advisable that ESD requiring around 1.5 h or more should be carried out under general anesthesia[21], patients were stratified into two groups according to procedure time (≤ 1.5 h or > 1.5 h) and then compared in terms of the following variables: age; sex; lifetime alcohol consumption; smoking habit; body weight (BW); tumor size (maximal diameter of the lesion); location (upper-third, middle-third, or lower-third of the stomach); gross morphological type (0- I / II a, 0- II b/ II c or combined type); tumor depth (mucosal or submucosal tumor); histological type (cancer or adenoma); ulcerative findings in the submucosal layer; and diazepam dosage.

Patients were also stratified into two groups according to diazepam dose: low-dose diazepam (≤ 17.5 mg, $n = 252$) and high-dose diazepam (> 17.5 mg, $n = 79$). These two groups were then compared in terms of age, sex, lifetime alcohol consumption, smoking habit, BW, use of anxiolytic agents, ASA classification, comorbidities (hypertension, diabetes mellitus, heart disease, respiratory disease, chronic renal failure, or liver cirrhosis), tumor size, tumor location, gross morphological type, tumor depth, histological type, ulcerative findings, type of resection (en bloc or piecemeal), postoperative bleeding, perforation, use of midazolam, and sedative-related adverse events such as oxygen desaturation (SpO_2 below 90%), hypotension (blood pressure below 90 mmHg), delayed awakening and paradoxical excitement.

Statistical analysis

Univariate analysis was performed using an unpaired *t*-test for numerical data and Fisher's exact test or the chi-squared test for categorical data. Variables that differed significantly between groups in univariate analysis were then subjected to multivariate analysis using a logistic regression model. All tests were two-sided, with values of $P < 0.05$ being considered statistically significant. All analyses were performed using SPSS software (SPSS, Chicago, IL, United States).

RESULTS

Comparison of clinicopathological features according to procedure time

The outcome of univariate analyses comparing variables according to the ESD procedure time (i.e., ≤ 1.5 h *vs* > 1.5 h) is outlined in Table 1. Significant differences were found between the two groups in relation to tumor size, location, ulcerative findings and diazepam dosage ($P < 0.001$, respectively). Specifically, mean diazepam dosage among patients with an ESD procedure time of > 1.5 h was 17.5 mg.

Comparison of clinicopathological features according to diazepam dose

Based on the above results, patients were divided into a low-dose (≤ 17.5 mg) diazepam group and a high-dose (> 17.5 mg) diazepam group. Results of univariate analyses of patient variables in relation to diazepam dosage are shown in Table 2. Significant differences in lifetime alcohol consumption and BW ($P = 0.032$ and $P = 0.006$, respectively) were found between the dosage groups. The results of univariate analyses for clinicopathological features of the lesion and clinical outcomes in relation to diazepam dosage are shown in Table 3. Significant differences in tumor size, location, ulcerative findings and resection style ($P = 0.001$ for each) were found between the two dosage groups.

Multivariate logistic analysis was performed including lifetime alcohol consumption, BW, tumor size, location and ulcerative findings in the prediction of the diazepam dosage. Each variable included in the model was shown to be independently associated with a need for high diazepam dosage (Table 4).

Patients were stratified into two groups on the basis of lifetime alcohol consumption (alcohol), using > 0.4 and ≤ 0.4 t as the strata. Finally, a second stratification was performed on the basis of BWs of > 60 kg and ≤

Table 1 Clinicopathological features of study subjects with a low (≤ 1.5 h) or high (> 1.5 h) procedure time

Variables	Procedure time ≤ 1.5 h (n = 180)	Procedure time > 1.5 h (n = 151)	P value
Age (yr) (mean ± SD)	69.9 ± 9.1	69.0 ± 9.6	NS
Sex (male/female)	136/44	125/26	NS
Lifetime alcohol consumption (t) (mean ± SD)	0.30 ± 0.50	0.37 ± 0.48	NS
Smoking habit (Brinkman index) (mean ± SD)	655.1 ± 777.7	563.0 ± 666.9	NS
Body weight (kg) (mean ± SD)	58.5 ± 10.9	60.1 ± 9.6	NS
Tumor size (mm) (mean ± SD)	13.3 ± 7.7	22.3 ± 16.0	< 0.001
Tumor location in stomach (U + M/L)	54/126	94/57	< 0.001
Gross morphological type (0-I / IIa *vs* 0-IIb / IIc *vs* combined)	92/68/20	76/66/9	NS
Tumor depth (mucosa/submucosa)	168/12	134/17	NS
Histological type (cancer/adenoma)	124/56	108/43	NS
Ulcerative findings, n (%)	2 (1.1)	30 (19.9)	< 0.001
Diazepam (mg) (mean ± SD)	9.9 ± 3.3	17.5 ± 7.8	< 0.001

SD: Standard deviation; NS: Not significant; U: Upper-third of the stomach; M: Middle-third of the stomach; L: Lower-third of the stomach.

Table 2 Clinical features of study subjects administered low- or high-dose of diazepam

Variables	Low-dose group (n = 252)	High-dose group (n = 79)	P value
Age (yr) (mean ± SD)	69.8 ± 9.1	68.3 ± 10.1	NS
Sex (male / female)	194/58	67/12	NS
Lifetime alcohol consumption (t) (mean ± SD)	0.30 ± 0.48	0.44 ± 0.52	0.032
Smoking habit (Brinkman index) (mean ± SD)	649.5 ± 767.7	497.8 ± 582.5	NS
Body weight (kg) (mean ± SD)	58.4 ± 10.3	62.0 ± 9.9	0.006
Anxiolytic agents (used/not used)	46/206	7/72	NS
ASA classification (ASA 1/ASA 2 / ASA 3)	48/151/53	20/47/12	NS
Comorbidities			
Hypertension, n (%)	127 (50.3)	39 (49.4)	NS
Diabetes mellitus, n (%)	44 (17.5)	11 (13.9)	NS
Heart disease, n (%)	58 (23.0)	18 (22.8)	NS
Respiratory disease, n (%)	30 (11.9)	4 (5.1)	NS
Chronic renal failure, n (%)	4 (1.6)	0 (0)	NS
Liver cirrhosis, n (%)	21 (8.3)	5 (6.3)	NS

SD: Standard deviation; NS: Not significant; ASA: American Society of Anesthesiologists.

60 kg. Thus, four subgroups were created and analyzed in relation to the diazepam dosage. The odds ratios of this logistic regression analysis are shown in Table 5. The combination of alcohol ≤ 0.4 t and BW ≤ 60 kg was defined as the standard subgroup. Odds ratios for the other three subgroups were found to increase in a stepwise fashion, with the greatest risk of high diazepam dose among patients with both alcohol > 0.4 t and BW > 60 kg (odds ratio = 4.52, 95% CI: 2.07 to 9.86).

Table 3 Clinicopathological features and clinical outcomes of subjects administered low- or high-dose of diazepam

Variables	Low-dose group (n = 252)	High-dose group (n = 79)	P value
Tumor size (mm) (mean ± SD)	15.4 ± 10.1	23.9 ± 18.2	< 0.001
Tumor location in stomach (U and M/L)	96/156	52/27	< 0.001
Gross morphological type (0-I / IIa *vs* 0-IIb / IIc *vs* combined)	129/100/23	39/34/6	NS
Tumor depth (mucosa/submucosa)	233/19	69/10	NS
Histological type (cancer/adenoma)	176/76	56/23	NS
Ulcerative findings, n (%)	14 (5.6)	18 (22.8)	< 0.001
Resection style (en bloc/piecemeal)	246/6	63/16	< 0.001
Postoperative bleeding, n (%)	1 (0.4)	1 (1.3)	NS
Perforation, n (%)	8 (3.2)	6 (7.6)	NS
Midazolam (added / not added)	43/209	20/59	NS

SD: Standard deviation; NS: Not significant; U: Upper-third of the stomach; M: Middle-third of the stomach; L: Lower-third of the stomach.

Table 4 Factors associated with the need for high doses of diazepam: Results of multivariate logistic analysis

Variable	P value	Odds ratio	95% CI
Lifetime alcohol consumption	0.041	1.74	1.02-2.97
Body weight	0.034	1.03	1.00-1.06
Tumor size	0	1.05	1.03-1.08
Location in stomach	0	2.87	1.61-5.12
Ulcerative findings	0.001	4.45	1.92-10.34

CI: Confidence interval.

Adverse events

Comparisons of adverse events according to diazepam dosage are included in Table 6. The incidence of paradoxical excitement was significantly higher in the high-dose diazepam group (P < 0.001). However, no other significant differences in adverse events were found.

DISCUSSION

This retrospective study revealed that gastric ESD can be performed in nearly 80% of patients under sedation achieved using a low dosage of diazepam. Patients with a long ESD procedure time were characterized by large-diameter tumors, lesions located in the upper- or middle-third of the stomach, and those accompanied by ulcerative findings. Outcomes found to be predictive of

Table 5 Comparison of need for high diazepam dose between subgroups stratified for lifetime alcohol consumption and body weight

Subgroup	Low-dose group (n = 252)	High-dose group (n = 79)	Odds ratio	95% CI
Alcohol > 0.4 t, BW > 60 kg	31	20	4.52	2.07-9.86
Alcohol > 0.4 t, BW ≤ 60 kg	38	17	3.13	1.43-6.88
Alcohol ≤ 0.4 t, BW > 60 kg	72	27	2.63	1.31-5.28
Alcohol ≤ 0.4 t, BW ≤ 60 kg	105	15	1	Referent

CI: Confidence interval. Alcohol: Lifetime alcohol consumption; BW: Body weight.

Table 6 Adverse events in patients administered a low *vs* high dose of diazepam

Variables	Low-dose group (n = 252)	High-dose group (n = 79)	P value
SpO2 < 90%, n (%)	70 (27.8)	18 (22.8)	NS
Blood pressure < 90 mmHg, n (%)	8 (3.2)	2 (2.5)	NS
Delayed awakening (flumazenil used/not used)	4/248	0/79	NS
Paradoxical excitement, n (%)	6 (2.4)	13 (16.5)	< 0.001

NS: Not significant.

a long ESD procedure time in the current study agreed with those previously reported by Goto *et al*[29]. To the best of our knowledge, no previous reports have confirmed that the sedative dose used during gastric ESD is increased in special patient groups (e.g., alcoholics or patients with higher BW). However, we found that a number of lesion-specific findings, as well as lifetime alcohol consumption and BW, were also associated with high-dose diazepam administration. In particular, lifetime alcohol consumption > 0.4 t and BW > 60 kg were additive risk factors for increased diazepam dosage. Specifically, patients with both a lifetime alcohol consumption > 0.4 t and a BW > 60 kg showed the greatest risk of needing a high diazepam dosage during ESD. While habitual alcohol consumption may increase the clearance of diazepam, the high lipid-solubility of diazepam may also result in rapid removal from the plasma and uptake by adipose tissue[30,31]. Therefore, when predicting diazepam dosages prior to starting gastric ESD, it is important to take into account not only the difficulty of the ESD procedure, but also the alcohol history and BW of the patient.

Although both respiratory and cardiovascular depression are common adverse events of diazepam administration, we encountered no serious events in the current study. For example, while oxygen saturation < 90% was observed in approximately 26% of patients, all recovered quickly in response to intraoperative supplemental oxygen administration and none required endotracheal intubation.

Debate is continuing regarding the proper depth of anesthesia required to perform lengthy endoscopic procedures such as ESD. We consider moderate sedation, which does not appear to cause respiratory depression, as the appropriate level of sedation. If the aim is to maintain the patient under moderate sedation with intermittent administration of a benzodiazepine, long-acting drugs such as diazepam are thought to be suitable in treatments requiring a relatively long time. Indeed, ESD procedures in almost all Japanese institutions are performed by an endoscopist who not only performs the ESD, but is also responsible for sedation during the operation. Due to a long half-life, diazepam is more suitable for intermittent than for continuous administration. Furthermore, intermittent administration in response to uncontrollable body movement is easy for a single operator to manage. The current analysis did not find any significant differences in the incidence of oxygen desaturation (SpO_2 below 90%) or hypotension (blood pressure below 90 mmHg) as a function of the administered diazepam dosage. These findings not only indicate the safety of diazepam, but also the suitability of its administration method.

Due to deep sedation in response to diazepam in the low-dosage diazepam group, flumazenil had to be administered to 4 patients (1.2%). Three of those patients had been coadministered 10 mg of midazolam, while another was an 85-year-old patient with a BW of only 42 kg. Kiriyama *et al*[17] reported that post-ESD recovery from sedation was faster with propofol than with midazolam. The present study did not perform scoring to investigate the recovery from sedation, but almost all patients were awake after returning to their hospital room following completion of the ESD procedure. Also, no cases showed carry-over of the sedative effect to the following morning. All patients who were administered flumazenil also showed rapid awakening, and no problems due to re-sedation were noted. Nevertheless, since ESD in Japan is currently performed as an inpatient treatment, as long as sufficient postoperative management is carried out, there may be no need for quick recovery of wakefulness.

Paradoxical excitement represents restless motion that occurs during diazepam administration. This reaction is reportedly caused, at least in part, by the toxicity of propylene glycol, an included diazepam solvent[32]. Propylene glycol is also a solvent that causes local irritation of veins. Some patients in the present study complained of transient vascular pain, but phlebitis was not seen in any patients. However, a notable increase in restlessness was observed with increasing diazepam dosages. Such reactions made the operation difficult to continue. Accordingly, in cases where preoperative prediction shows a strong possibility that a large dose of diazepam will be required, a different approach to sedation may be advisable. Examples include continuously administering propofol or dexmedetomidine from the start of the

operation, a technique that has recently been reported as useful during ESD[17,33].

The present study has several limitations. First, data generated from only a single hospital were reviewed retrospectively. Second, the decision to administer additional diazepam was left up to the operator, and the timing of such administration was not consistent across patients. However, the most important aspect of this study was the evaluation of the suitability of intermittent administration of diazepam prior to ESD. Further studies at multiple institutions should be conducted using different benzodiazepines and concomitant drugs, with different methods of administration.

In conclusion, among patients who are predicted to require only a low dosage of diazepam during ESD, intermittent administration of diazepam for sedation during gastric ESD will enable safe completion of the surgery. The need for high-dose diazepam can be expected in patients with lifetime alcohol consumption > 0.4 t, BW > 60 kg, or requiring a technically difficult ESD procedure. Given the present results, further randomized trials performed in a prospective manner with clear inclusion criteria and a clear injection protocol should be conducted for such patients.

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COMMENTS

Background

Endoscopic submucosal dissection (ESD) is a curative treatment for gastric epithelial neoplasia. Many cases of gastric epithelial neoplasia occur in elderly patients, who show increased sensitivity to sedatives and a higher risk of adverse reactions. Suitable methods for the administration of sedatives during ESD thus need to be established.

Research frontiers

This study can help us to understand the diazepam dosage required during ESD for gastric epithelial neoplasia and the characteristics of and adverse events encountered by patients administered high-dose diazepam.

Innovations and breakthroughs

Diazepam is the least potent injectable benzodiazepine sedative, with a long history of clinical use. However, administration methods have yet to be clearly established for safe and effective sedative use during gastric ESD procedures.

Applications

The results have demonstrated that intermittent administration of diazepam enabled safe completion of gastric ESD except for patients who are alcohol abusers or obese, or those with complicated lesions.

Peer review

This retrospective study investigated risk factors and adverse events related to high-dose diazepam administration during ESD for gastric neoplasias. Based on the present results, further randomized trials performed prospectively with clear inclusion criteria and a clear injection protocol should be conducted.

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ORIGINAL ARTICLE

Identification of gastric cancer risk markers that are informative in individuals with past *H. pylori* infection

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Abstract

Background Epigenomic damage induced by *Helicobacter pylori* infection is accumulated in gastric mucosae before the development of malignancy. In individuals without current *H. pylori* infection, DNA methylation levels of specific CpG islands (CGIs) are associated with gastric cancer risk. Because risk estimation in individuals with past infection is clinically important, we here aimed to identify the risk markers that reflect epigenomic damage induced by *H. pylori* infection, and that are informative in these individuals.

Methods Gastric mucosae were obtained from 55 gastric cancer patients (GC-Pt) (21 with current infection and 34 with past infection) and 55 healthy volunteers (HV) (7 never-infected, 21 with current infection, and 27 with past infection). Hypermethylated CGIs were searched for by methylated DNA immunoprecipitation-CGI microarray, and methylation levels were analyzed by quantitative methylation-specific polymerase chain reaction (PCR).

Results By microarray analysis of a pool of three samples from GC-Pt with past infection and another pool of samples from HV with past infection, 15 hypermethylated CGIs in the former pool were isolated. Seven of them had significantly higher methylation levels in GC-Pt with past infection ($n = 10$) than in HV with past infection ($n = 10$) ($P < 0.001$). In a validation cohort (21 GC-Pt with past infection and 14 HV with past infection), the seven new markers had large areas under the receiver-operating characteristic curves (0.78–0.84) and high odds ratios (12.7–36.0) compared with two currently available markers (0.60–0.65, 5.0–5.7).

Conclusions We identified seven novel gastric cancer risk markers that are highly informative in individuals with past infection.

Keywords Carcinogenesis · DNA methylation · Gastric cancer · *Helicobacter pylori*

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Introduction

Early detection of cancer is critically important to reduce its morbidity and mortality, and early detection can be achieved by identifying individuals at high risk of developing cancers. In the risk estimation of gastric cancers, a history of *Helicobacter pylori* infection, which increases gastric cancer risk 2.2- to 21-fold [1–4], plays the major role, but the vast majority of individuals with a history of *H. pylori* infection do not develop gastric cancers. Also, gene polymorphisms associated with gastric cancers have been identified, and they have been shown to confer odds ratios (ORs) mostly between 1.0 and 2.0 [5, 6]. To obtain

clinically useful risk markers, we have to develop markers that are informative even in individuals with a history of *H. pylori* infection and that confer higher ORs.

Recently, we showed that *H. pylori* infection induces epigenomic damage, especially aberrant DNA methylation, in gastric mucosae [7]. DNA methylation levels of specific CpG islands (CGIs) were very high in the gastric mucosae of individuals with active *H. pylori* infection irrespective of gastric cancer risk, and decreased to certain levels after *H. pylori* was eradicated [8]. Importantly, these methylation levels in individuals without active *H. pylori* infection were correlated with gastric cancer risk [7, 9]. It is considered that aberrant DNA methylation is induced both in gastric stem cells and in non-stem cells, that methylation induced in stem cells will remain even after *H. pylori* eradication, and that methylation levels in individuals without current *H. pylori* infection reflect gastric cancer risk (degree of the epigenetic field defect) [10].

The correlation between methylation levels and gastric cancer risk has been analyzed in individuals without current *H. pylori* infection [7, 9]. Based on the data in our previous study [7], currently available methylation risk markers, *FLNc* and *THBD*, have ORs of 4.2–7.0 to detect gastric cancer patients (GC-Pt) among such individuals. However, individuals without current *H. pylori* infection indeed consist of never-infected individuals and those with past infection, and risk estimation is important in individuals with past infection.

In this study, we aimed to identify gastric cancer risk markers that reflect epigenomic damage induced by *H. pylori* infection, and that are informative in individuals with past infection.

Materials and methods

Tissue samples and determination of *H. pylori* infection status

Fifty-five healthy volunteers (HV) with endoscopic findings of no malignancy were recruited, with written informed consents, on the occasion of a gastric cancer screening program, with the approval of the institutional review board. Fifty-five GC-Pt who had undergone curative endoscopic submucosal dissection (ESD) of a well-differentiated adenocarcinoma in the non-cardia according to the Japanese classification of gastric carcinoma [11] were also recruited, with written informed consents, with the approval of the Institutional Review Board. Gastric mucosae were collected by endoscopic biopsy of the antrum. The biopsy specimens were frozen in liquid nitrogen immediately after biopsy, and stored at −80°C until DNA extraction. High molecular weight DNA was extracted by the phenol/chloroform method.

Current *H. pylori* infection was analyzed by a serum anti-*H. pylori* IgG antibody test (SRL, Tokyo, Japan) in HV and by urea breath test (Otsuka Pharmaceutical, Tokushima, Japan) in GC-Pt. Also, the presence of current or past *H. pylori* infection was detected by the endoscopic presence of atrophic gastritis in the antrum, because atrophic change induced by *H. pylori* infection arises in the antrum in 83% of individuals with *H. pylori* infection [12] and remains in all individuals who have had *H. pylori* eradication therapy [13]. "Never-infected individuals" were defined as those who were negative for *H. pylori* analysis and did not have atrophic gastritis in the antrum. "Individuals with current infection" were defined as those who were positive for *H. pylori* analysis. "Individuals with past infection" were defined as those who were negative for *H. pylori* analysis and had atrophic gastritis in the antrum.

Methylated DNA immunoprecipitation-CGI microarray analysis

Methylated DNA immunoprecipitation (MeDIP)-CGI microarray analysis was performed as previously described [14, 15]. Briefly, 5 μg of genomic DNA was immunoprecipitated with an anti-5-methylcytidine antibody (Diagnode, Liége, Belgium), and the precipitated DNA and the input DNA were labeled with cyanin (Cy) 5 and Cy3, respectively. A human CGI oligonucleotide microarray (Agilent Technologies, Santa Clara, CA, USA) was hybridized with the labeled probes and scanned with an Agilent G2565BA microarray scanner (Agilent Technologies). Scanned data were processed with Feature Extraction Software Version 9.1 (Agilent Technology) and Agilent G4477AA ChIP Analytics 1.3 software. The signal of a probe was converted into a "Me value", which represented the methylation level as a value from 0 (unmethylated) to 1 (methylated). Differentially methylated regions were detected by comparison between the Me values of two samples, and data were visualized in the UCSC Genome Browser (http://genome.ucsc.edu/) on NCBI36/hg18 assembly (National Center for Biotechnology Information, Bethesda, MD, USA).

Sodium bisulfite modification and quantitative methylation-specific polymerase chain reaction

Fully methylated DNA and fully unmethylated DNA were prepared by methylating genomic DNA with *SssI* methylase (New England Biolabs, Beverly, MA, USA) and by amplifying genomic DNA with the GenomiPhi amplification system (GE Healthcare, Buckinghamshire, UK), respectively. Bisulfite modification was performed using 1 μg of *Bam*HI-digested genomic DNA, and the modified

DNA was suspended in 40 μl of Tris–ethylenediamine tetraacetic acid (EDTA) buffer [16]. An aliquot of 2 μl of sodium bisulfite-treated DNA was used in one reaction of quantitative methylation-specific polymerase chain reaction (PCR; qMSP).

qMSP was performed using primer sets specific to methylated and unmethylated sequences (Supplementary Table 1), SYBR® Green I (BioWhittaker Molecular Applications, Rockland, ME, USA), and an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The number of molecules in a sample was determined by comparing its amplification with those of standard DNA that contained known numbers of molecules (10^1–10^9 molecules). Standard DNA was prepared by purifying the PCR products using the Wizard SV Gel and PCR Clean-Up System (Promega, Fitchburg, WI, USA). The methylation level was calculated as the fraction of methylated (M) molecules in the total number of DNA molecules (number of M molecules + number of unmethylated molecules). The percentage of methylated reference (PMR) was calculated as the fraction of the methylated reference {(number of M molecules in a sample)/(number of *Alu* repeat sequences in a sample)}/{(number of M molecules in *SssI*-treated DNA)/(number of *Alu* repeat sequences in *SssI*-treated DNA)} [17].

Statistical analysis

Differences in mean methylation levels or PMR were analyzed by the Student's *t*-test. The receiver-operating characteristic (ROC) curve was drawn, and the area under the curve (AUC) and OR were analyzed by binomial distribution and binomial logistic regression analysis, respectively. All the analysis was performed using PASW statistics (SPSS, Chicago, IL, USA), and the results were considered significant when *P* values of less than 0.05 were obtained by two-sided tests.

Results

Isolation of hypermethylated CGIs in GC-Pt compared with HV in individuals with past *H. pylori* infection

A pool of three samples from HV with past infection and another pool of three samples from GC-Pt with past infection were analyzed by MeDIP-CGI microarray analysis. CGIs that were hypermethylated in the latter group compared with the former group were selected as follows: (1) Me value in the latter pool was higher than that in the former pool by 0.2 or more, (2) Me value in the former pool was lower than 0.4, and (3) criteria (1) and (2) were satisfied in three consecutive probes. A total of 15 CGIs were isolated by these criteria (Table 1), and representative data around CGI #5 are shown in Fig. 1.

From the 15 CGIs, those differentially methylated in a screening set, which consisted of 10 HV with past infection and 10 GC-Pt with past infection, were searched for by evaluating PMRs by qMSP (Supplementary Table 2). Seven CGIs (#1 to #7; Table 1), distributed on various chromosomes, were methylated at significantly higher

Table 1 CGIs identified by MeDIP-CGI microarray

CGI no.	Gene symbol	Name	Chromosomal position	Location around a gene
#1	*EMX1*	Empty spiracles, homeobox 1	2p13.2	Intron 1
#2	*miR663*	MicroRNA 663	20p11.1	Overlap
#3	*NKX6-1*	NK6, homeobox 1	4q21.23	Intron 1
#4	*OTP*	Orthopedia homeobox	5q13.3	Downstream
#5	*OPLAH*	5-Oxoprolinase (ATP-hydrolysing)	8q24.3	Downstream
#6	*CYP1B1*	Cytochrome P450, family 1, subfamily B, polypeptide 1	2p22.2	Exon 1
#7	*NEFM*	Neurofilament, medium polypeptide	8p21	Exon 1
#8	*PMF1*	Polyamine-modulated factor 1	1q22	Intron 1
#9	*BDNF*	Brain-derived neurotrophic factor	11p14.1	Intron 1
#10	*SSTR5*	Somatostatin receptor 5	16p13.3	Promoter
#11	*MYO1D*	Myosin ID	17q11.2	Intron 1
#12	*CAMK2N2*	Calcium/calmodulin-dependent protein kinase II inhibitor 2	3q27.1	Promoter
#13	*GATA4*	GATA binding protein 4	8p23.1	Promoter
#14	*NFATC1*	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	18q23	Promoter
#15	*ANKRD9*	Ankyrin repeat domain 9	14q32.31	Exon 1

CGI CpG island, *MeDIP* methylated DNA immunoprecipitation

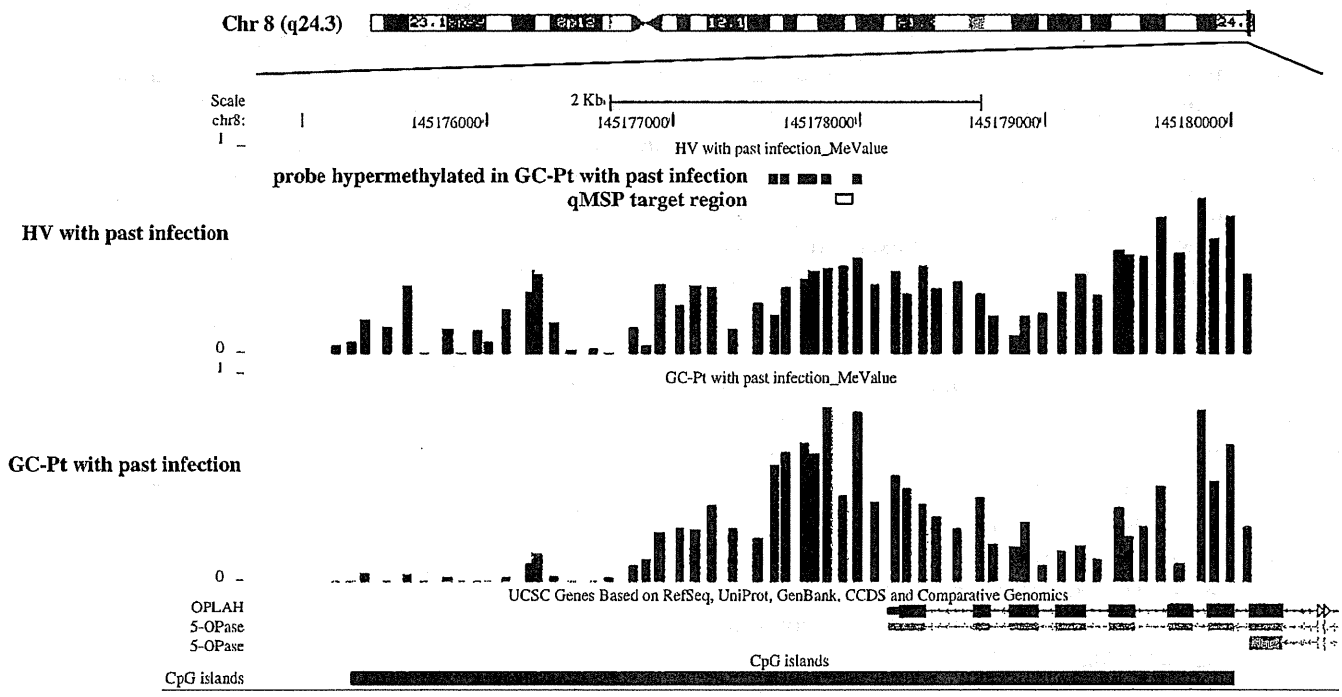

Fig. 1 Data of methylated DNA immunoprecipitation-CpG island (MeDIP-CGI) microarray analysis in the genomic region around CGI #5. Methylation levels were assessed by Me values, and the Me values of the two pools were visualized by the UCSC Genome Browser (http://genome.ucsc.edu/) for a genomic region (from nt. 145,174,733 to nt. 145,180,586 on chromosome 8 in NCBI36/hg18 assembly). *Vertical bars* show Me values of individual probes. *Closed boxes* above the Me values indicate the differentially methylated probes. Quantitative methylation-specific polymerase chain reaction (*qMSP*) primers were designed in the area shown by the *open box*. *HV* healthy volunteers, *GC-Pt* gastric cancer patients

levels in GC-Pt than in HV ($P < 0.05$). Relative positions against a gene also varied—two CGIs being located in exon 1, two in intron 1, two 300 bp downstream of the annotated end, and one overlapping with *pre-microRNA 663*.

Validation of the usefulness of the seven markers

The usefulness of the seven CGIs was validated by qMSP analysis of an independent set of samples (Fig. 2). The validation set consisted of seven never-infected HV (Group [G] 1), 21 HV with current infection (G2), 14 HV with past infection (G3), 21 GC-Pt with current infection (G4), and 21 GC-Pt with past infection (G5) (Supplementary Table 3). For comparison, two currently available markers (*FLNc* and *THBD*) were also analyzed. In the individuals with past infection (G3 and G5), the seven CGIs had levels that were 2.8-, 1.5-, 3.8-, 2.3-, 2.5-, 1.8-, and 3.8-fold, respectively, higher in G5 than in G3 ($P < 0.01$). *FLNc* tended to have a higher level in G5 than in G3 ($P = 0.087$), but *THBD* did not show any significant difference ($P = 0.341$). These data showed that the methylation levels of all the seven CGIs had the power of cancer risk estimation even in individuals with past infection.

In the HV, methylation levels in G2 were much higher than those in G1 ($P < 0.05$), but those in G3 were lower than those in G2. This observation supported the model that active infection by *H. pylori* induces methylation potently in non-stem cells, in addition to stem cells, and that methylation levels will eventually decrease after *H. pylori* infection has been eradicated. Also, methylation levels in G3 were significantly higher (four of the seven CGIs, $P < 0.05$) or tended to be higher than those in G1. This observation again supported the model that methylation induced in stem cells will remain even after *H. pylori* infection is eradicated.

Power of the seven CGIs as gastric cancer risk markers

AUCs to detect individuals in G5 were calculated using individuals in G3 and G5 (Table 2; Fig. 3). AUCs for the seven CGIs ranged between 0.78 and 0.84 and were significantly larger than 0.5 ($P < 0.01$). In contrast, the AUCs for the two currently available markers were 0.69 (95% CI 0.51–0.87) and 0.65 (95% CI 0.45–0.84), respectively, and were not significantly different from 0.5. Using optimal cut-off values obtained by the ROCs, ORs for the seven CGIs were calculated to be 12.7–36.0 (Table 2). ORs for