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RESEARCH REPORT

Risk factors associated with Barrett's epithelial dysplasia

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Abstract

AIM: To elucidate risk factors associated with dysplasia of short-segment Barrett's esophagus (BE).

METHODS: A total of 151 BE patients who underwent endoscopic examination from 2004 to 2008 in Aoyama Hospital, Tokyo Women's Medical University, Japan and whose diagnosis was confirmed from biopsy specimens were enrolled in the study. BE was diagnosed based on endoscopic findings of gastric-appearing mucosa or apparent columnar-lined esophagus proximal to the esophagogastric junction. Dysplasia was classified into three grades - mild, moderate and severe - according to the guidelines of the Vienna Classification System for gastrointestinal epithelial neoplasia. Anthropometric and biochemical data were analyzed to identify risk factors for BE dysplasia. The prevalence of *Helicobacter pylori* (*H. pylori*) infection and the expression of *p53* by immunohistological staining were also investigated.

RESULTS: Histological examination classified patients into three types: specialized columnar epithelium (SCE) (n = 65); junctional (n = 38); and gastric fundic (n = 48). The incidence of dysplasia or adenocarcinoma from BE of the SCE type was significantly higher than that of the other two types ($P < 0.01$). The univariate analysis revealed that sex, *H. pylori* infection, body weight, *p53* overexpression, and low diastolic blood pressure (BP) were associated with BE dysplasia. In contrast, body mass index, waist circumference, metabolic syndrome complications, and variables related to glucose or lipid metabolism were not associated with dysplasia. Multivariate logistic analysis showed that overexpression of *p53* [odds ratio (OR) = 13.1, P = 0.004], *H. pylori* infection (OR = 0.19, P = 0.066), and diastolic BP (OR = 0.87, P = 0.021) were independent risk factors for epithelial dysplasia in BE patients with the SCE type.

CONCLUSION: Overexpression of *p53* is a risk factor for dysplasia of BE, however, *H. pylori* infection and diastolic BP inversely associated with BE dysplasia might be protective.

Key words: Barrett's esophagus; Dysplasia; *Helicobacter pylori*; *p53*; Risk factors

Core tip: Barrett's esophagus (BE) is known to be a precancerous state of adenocarcinoma will become common in Asian countries, therefore, it is important to establish a high-risk group or strategy for screening or follow-up of BE. We present here the results of univariate and multivariate analysis to identify variables associated with dysplasia of BE. *p53* expression in immunohistochemistry was associated with dysplasia, and *Helicobacter pylori* infection and high diastolic blood pressure may act as protective factors against dysplastic change of BE. These three factors may be candidates to establish a high-risk group for esophageal adenocarcinoma.

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INTRODUCTION

Barrett's esophagus (BE) is defined as a condition in which normal squamous mucosa is replaced by columnar epithelium. This intestinal metaplasia of the distal esophagus is considered to be a premalignant condition where metaplasia may progress to dysplasia and subsequently to adenocarcinoma[1-6]. BE is generally regarded as a complication of chronic and severe gastroesophageal reflux disease (GERD). Elevation of the intra-abdominal pressure by obesity is a factor contributing to GERD, suggesting that obesity is a risk factor for BE[7-10]. GERD and BE appear to be metabolic syndrome (MS)-related complications, given that waist circumference, obesity, and body mass index (BMI) are associated with GERD[11-16].

Moreover, *Helicobacter pylori* (*H. pylori*) infection may play a key role in suppression of BE. Two main inhibiting roles for development of BE have been postulated in *H. pylori* infection: *H. pylori*-induced atrophic gastritis resulting in less gastric acid secretion; and neutralization of the gastric acid by ammonia produced by *H. pylori* independently of gastric atrophy[17-23]. Cag-A positive *H. pylori* infection is strongly associated with a reduced risk of esophageal adenocarcinoma, and the association is independent of gastric atrophy, suggesting the involvement of a mechanism other than reduced acidic gastric reflux[24-26]. In Japan, the prevalence of *H. pylori* is declining and it can be easily eradicated; however, it remains uncertain whether the incidence of BE will increase or decrease as a consequence of the low prevalence of *H. pylori* infection[27,28].

BE is characterized by three types of columnar epithelium, namely, cardiac type (junctional type), fundic type, and intestinal metaplasia type [specialized columnar epithelium type (SCE) type]. It has been shown that there is a high incidence of adenocarcinoma in the distal esophagus arising from SCE in patients with BE[29,30].

BE is classified as either long-segment type (length $\geqslant$ 3 cm) or short-segment type (length < 3 cm). In western countries, long-segment Barrett's esophagus (LSBE) is more prevalent, while short-segment Barrett's esophagus (SSBE) is more common, and the incidence of adenocarcinoma arising from SSBE is steadily increasing in Japan[27,28,31-33].

Several studies have shown that most patients with BE do not progress to cancer, although some do[1-3,29,30]. Thus, it is important to determine how BE progresses to dysplasia and adenocarcinoma and to identify the type of BE patients who may have a possibility of malignant transformation in SCE. It has been reported that central adiposity, metabolic syndrome, and high BMI are associated with BE and adenocarcinoma[10-16]. In this study, we investigated the risk factors associated with BE dysplasia.

MATERIALS AND METHODS

Study population

A total of 151 patients (105 male, 46 female) with histologically diagnosed BE were enrolled. All cases were incident cases and enrolled in a consecutive series from April 2004 to March 2008. Patients who had received antibiotics, steroids, or nonsteroidal anti-inflammatory drugs were excluded from the study. Patients were also excluded if they had peptic ulcer, underwent partial gastrectomy, consumed alcohol excessively, or had comorbid diseases such as liver cirrhosis and uremia. Written informed consent was obtained from all patients.

Endoscopic examination

BE was diagnosed based on endoscopic findings of gastric-appearing mucosa or apparent columnar lined esophagus proximal to the esophagogastric junction. The esophagogastric junction was defined as the pinch at the end of the tubular esophagus coinciding with the proximal margin of the gastric folds of the hiatal hernia. BE was defined as columnar mucosa proximal to the distal ends of esophageal longitudinal palisading vessels according to the Japanese Society of Esophageal Disease[34,35]. These veins are visible endoscopically when a conscious patient takes a deep breath.

SSBE was defined as an epithelium length < 3 cm and LSBE as an epithelium length > 3 cm, as described previously[1,2,29,30] The length of BE were measured by measuring forceps (Olympus, Tokyo, Japan).

When abnormal columnar mucosa characteristics such as erosions, red flares, elevated regions, or mucosal breaks were observed between the proximal limit of the gastric folds and squamous epithelium, we detected metaplastic change by chromoendoscopy and staining mucosa with crystal violet (Figure 1A, B). For chromoendoscopy 200000 U pronase (Pronase MS; Kaken Pharmaceutical Co., Matsumoto, Japan) dissolved in 300 mL warm water was sprayed around the esophagogastric junction area with a spray tube, and a 0.03% solution of crystal violet was applied to the same area. A few minutes later, the sprayed area was washed thoroughly with water. When mucosa showing a tubular or villous pit pattern, which is typical of SCE in BE, was observed in the esophagogastric junction, we performed a targeted biopsy in that area. BE was confirmed by histological findings from biopsy specimens in all patients.

Histology

All biopsy specimens were fixed in formalin, embedded in paraffin, sectioned, mounted on slides and then stained with hematoxylin and eosin using standard techniques. Dysplasia was classified into three grades: mild, moderate and severe, according to the guidelines of the Vienna Classification System for gastrointestinal epithe-

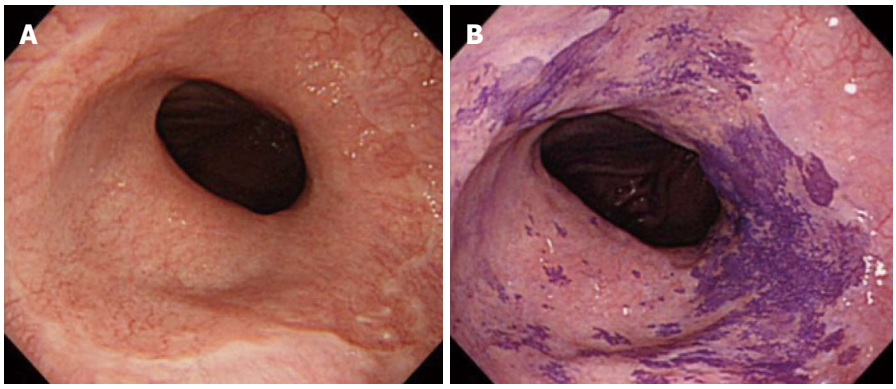

Figure 1 Barrett's esophagus stained by crystal violet. A: Regular observation of Barrett's esophagus; B: Staining with crystal violet in the same region.

lial neoplasia[17,36]. To perform immunohistological staining of p53, an anti-human p53 antibody (DO-7 mouse monoclonal antibody, IR616; Dako, Denmark) was used according to the manufacturer's protocol. The expression level of p53 protein was determined and graded based on the intensity of nuclear staining in columnar cells as follows: no staining (-), positive nuclear staining in 5%-10% of cells (+), and positive nuclear staining in > 10% of cells (++), according to the work of Keswani *et al*[37] (Figure 2). All biopsy specimens were examined by an experienced gastrointestinal pathologist.

H. pylori infection

The presence of gastric *H. pylori* was determined based on the results of Giemsa and/or Steiner's silver staining in a minimum of three gastric surveillance biopsies (one each obtained from the antral greater curvature, greater curvature of the mid to distal body, and lesser curvature in the proximal body). *H. pylori* colonization was assessed by an experienced pathologist blinded to the clinical data. Patients who were not confirmed *H. pylori* infection by using above histological analysis were retested by other methods. We confirmed *H. pylori* negativity by combination of serum HP-specific antibody test with 13C-urea breath test or *H. pylori* antigen test in the stools.

Anthropometry and blood pressure

The body weights of patients, while not wearing heavy outdoor clothing or shoes, was measured to the nearest 0.1 kg using a digital scale. Height (barefoot) was measured using a portable stadiometer. Waist circumference was measured to the nearest 0.1 cm using a plastic tape just above the umbilical portion while standing in a relaxed position after gentle expiration. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured with a mercury sphygmomanometer on each arm after at least 10 min of rest.

Definition of MS and biochemical analysis

MS was diagnosed according to the criteria set out by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome[38]: central obesity (waist circumference $\geqslant$ 85 cm Japanese men, $\geqslant$ 90 cm Japanese women) plus any two of the following; raised triglycerides $\geqslant$ 150 mg/dL or specific treatment for this lipid abnormality; reduced high density lipoprotein (HDL)-cholesterol < 40 mg/dL in men and women; raised blood pressure (systolic $\geqslant$ 130 mmHg or diastolic $\geqslant$ 85 mmHg) or treatment of hypertension; fasting glucose $\geqslant$ 110 mg/dL or previously diagnosed type 2 diabetes mellitus.

After a 12-h overnight fast, venous blood samples were taken for the standard biochemical data.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0 (SPSS, Tokyo, Japan). Results for continuous variables were expressed as mean ± SD for each subject group. The statistical difference was determined by two-sided Student's *t* test (for equal variance cases) or Welch's *t* test (for unequal variance cases). Non-normally distributed variables were compared by the Mann-Whitney *U* test. Variables given as proportions were compared using the χ^2 test. The relationships between risk factors and dysplasia including adenocarcinoma of BE were examined by multivariate logistic regression analysis. $P < 0.05$ was considered to be statistically significant. Differences in mean laboratory data and anthropometric data across three categories were evaluated using one-way analysis of variance (ANOVA).

RESULTS

Endoscopic findings of BE by crystal violet staining

Crystal violet staining was performed when we recognized BE during routine endoscopic examination. The intestinal metaplastic lesion was stained with a violet color, resulting in easy recognition of the targeted biopsy (Figure 1A, B).

Dysplasia in SCE-type BE

The average age of the 151 BE patients was 62.9 ± 10.6 years and there were 105 men to 46 women (ratio 2.3:1). The demographic characteristics of the patients according to pathological classification are shown in Table 1. BE patients were classified into three categories: SCE type (n = 65), junctional type (n = 38), and gastric fundic

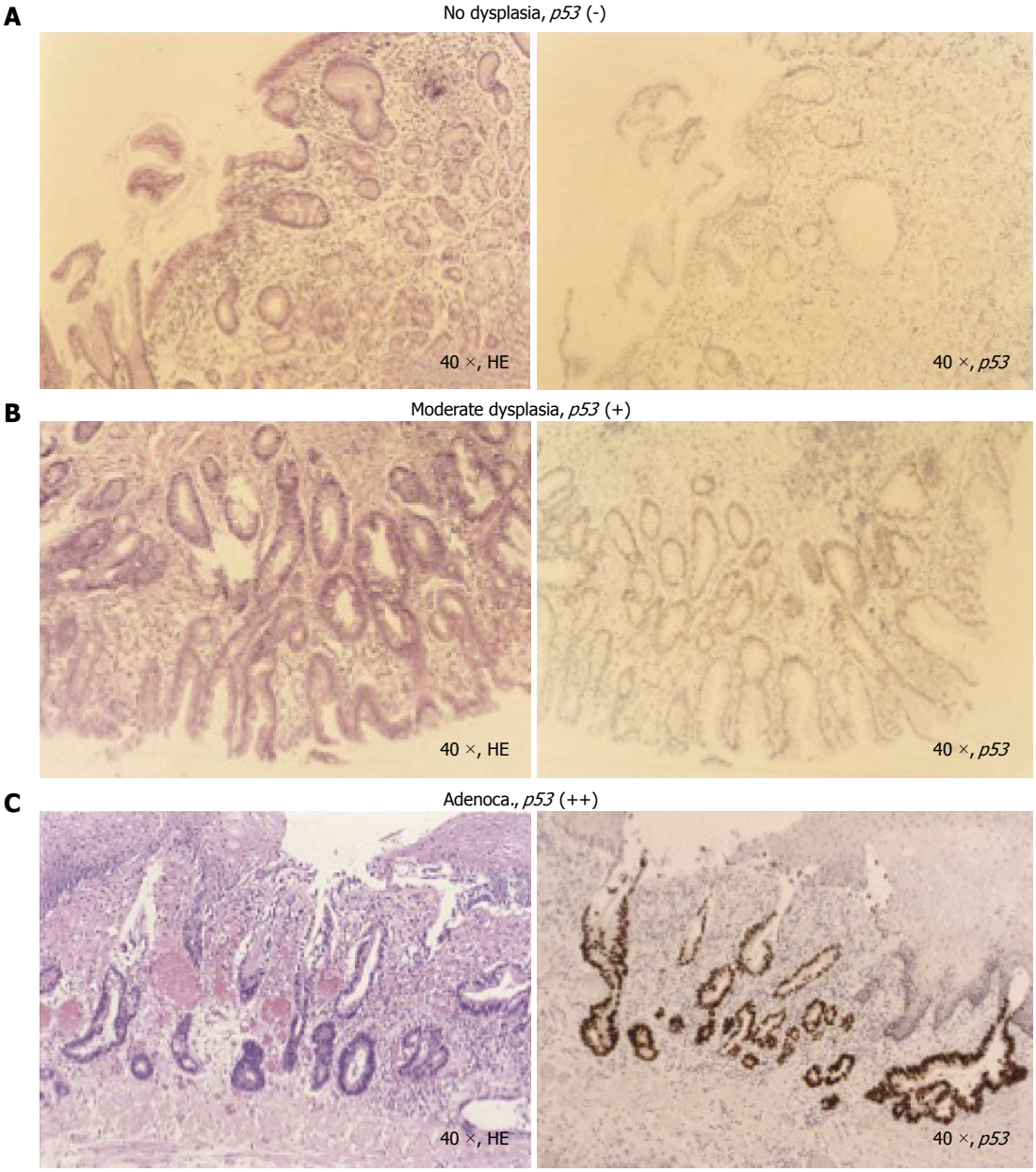

Figure 2 Immunostaining of *p53*. The upper panel shows hematoxylin and eosin staining and the lower panel shows immunostaining of *p53* using the identical sample. A: (-), no *p53* expression; B: (+), moderate *p53* expression characterized by positive nuclear staining in 5%-10% of cells; C: (++), high *p53* expression characterized by positive nuclear staining in > 10% of cells.

Table 1 Characterization of the three types of Barrett's esophagus

	Specialized columnar			*P* value
	Epithelium type (*n* = 65)	Junctional type (*n* = 38)	Gastric fundic type (*n* = 48)	
Sex	52/13	10/28	25/23	0.005
Age (yr)	62.9 ± 10.6	65.0 ± 8.2	58.8 ± 11.9	0.07
Dysplasia	20/65 (30.8%)	3/38 (7.9%)	2/48 (4.2%)	0.003

type (n = 48), and the incidence of dysplasia in these three groups was 30.8% (20/65), 7.9% (3/38) and 4.2% (2/48), respectively. The ratio of dysplasia in patients with SCE-type BE was significantly higher than in patients with junctional- and gastric-fundic-type BE (P = 0.02 and P = 0.002, respectively).

Variables associated with dysplasia in SCE-type BE

We focused on the SCE type of BE because of the high rate of dysplastic change associated with this condition, as shown in Table 1. We compared variables between SCE-type BE patients with and without dysplasia (Table 2). *H. pylori* infection, *p53* overexpression (Figure 2), body weight, and diastolic BP were identified as risk factors strongly associated with dysplastic change. In contrast, BMI, waist circumference, MS complications, and variables related to glucose or lipid metabolism were not associated with dysplasia. We then conducted multivariate logistic analysis of those variables that showed low P

Table 2 Univariate analysis of variables associated with Barrett's epithelial dysplasia

	Normal (n = 45)	Dysplasia (n = 20)	P value	OR	95%CI
Sex (male/female)	Dec-33	19/1	0.073	0.145	0.017-1.202
Age (yr)	65.0 ± 9.8	64.2 ± 11.3	0.730	0.991	0.940-1.044
Length of BE (SSBE/LSBE)	Feb-43	18/2	0.402	2.389	0.312-18.294
H. pylori infection rate	34/9 (2)	7/12 (1)	0.002[1]	0.154	0.047-0.506
p53 positive rate	4/41	13/7	< 0.001[1]	19.036	4.800-75.499
Body weight (kg)	61.0 ± 9.7	68.2 ± 10.6	0.019	1.081	1.013-1.154
BMI (kg/m^2)	23.1 ± 2.8	23.7 ± 3.2	0.451	1.074	0.891-1.295
Waist circumference (cm)	86.9 ± 7.9	88.4 ± 9.0	0.585	1.022	0.945-1.105
GERD	19/26	9/11	0.835	1.120	0.387-3.235
Hypertension (BP > 130/85)	29/14 (2)	9/10 (1)	0.094	0.395	0.133-1.172
Systolic BP (mmHg)	127 ± 16	121 ± 16	0.264	0.980	0.946-1.015
Diastolic BP (mmHg)	76 ± 10	69 ± 8	0.009	0.907	0.843-0.976
Diabetes	14/25 (6)	10/9 (1)	0.188	2.071	0.701-6.124
Fasting blood glucose (mg/dL)	118 ± 32	111 ± 19	0.447	0.992	0.971-1.013
HbA1c (%)	5.6 ± 1.0	5.5 ± 0.7	0.804	0.919	0.470-1.798
Dyslipidemia	24/19 (2)	11/8 (1)	0.322	0.576	0.193-1.715
TG (mg/dL)	144 ± 58	139 ± 98	0.807	0.999	0.991-1.007
TC (mg/dL)	202 ± 30	189 ± 33	0.141	0.987	0.969-1.005
HDL-C (mg/dL)	55 ± 13	53 ± 9	0.850	0.996	0.950-1.043
LDL-C (mg/dL)	118 ± 26	107 ± 30	0.152	0.985	0.964-1.006
Fatty liver	17/13 (15)	6/8 (6)	0.395	0.574	0.159-2.066
γ-GT (U/L)	55 ± 48	59 ± 77	0.781	1.001	0.992-1.011
AST (IU/L)	24 ± 8	25 ± 10	0.769	1.009	0.948-1.075
ALT (IU/L)	24 ± 15	24 ± 12	0.906	0.998	0.958-1.039
hs-CRP (mg/dL)	0.107 ± 0.142	0.101 ± 0.116	0.890	0.706	0.005-98.830
MS	15/14 (16)	6/8 (6)	0.586	0.700	0.194-2.530

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BP: Blood pressure; GT: Glutamyl transpeptidase; HbA1c: Hemoglobin A1c; HDL-C: High-density lipoprotein cholesterol; hs-CRP: High sensitivity C-reactive protein; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; TC: Total cholesterol; *H. pylori*: *Helicobacter pylori*.

Table 3 Multivariate analysis of variables associated with Barrett's epithelial dysplasia

Risk factor	P value	OR	95%CI
p53	0.004	13.107	2.275-75.504
H. pylori	0.066	0.187	0.031-1.116
Diastolic BP	0.021	0.874	0.780-0.980

H. pylori: *Helicobacter pylori*; BP: Blood pressure.

values in the univariate analysis shown in Table 2; namely, sex, *H. pylori* infection, body weight, *p53* overexpression, and low diastolic BP. Among these these variables, *p53* overexpression, *H. pylori* infection, and low diastolic BP were independent risk factors associated with dysplasia complicated in patients with BE of the SCE type (Table 3). The P value of *H. pylori* infection was 0.066 (not less than 0.05), but the odds ratio was 0.187, which seemed to give a relatively strong effect on the association with Barrett epithelial dysplasia. Thus, we included it in Table 3.

Risk factors associated with progression of SCE from nondysplastic epithelium to low-grade and high-grade dysplasia

We assesed the linearity of the relationship between risk factors and progression. We classified dysplasia into three groups: no dysplasia (n = 45), low-grade dysplasia (n = 14), and high-grade dysplasia (n = 6) including adenocarcinoma (Table 4). Based on ANOVA, six variables were significantly associated with alteration of SCE from non-dysplasia to high-grade dysplasia: length of BE, *H. pylori* infection, *p53* overexpression, body weight, GERD, and low diastolic BP. Only two of these six variables showed a linear correlation with alteration to high-grade dysplasia; that is, *H. pylori* infection and *p53* overexpression (Table 4).

Correlation between p53 expression and progression of SCE from non-dysplasia to low- and high-grade dysplasia

Given the strong association observed between *p53* overexpression and dysplasia seen in the multivariate logistic analysis (Table 3), we analyzed the level of *p53* expression and its association with progression of nondysplastic SCE to low- and high-grade dysplasia including adenocarcinoma. The expression of *p53* was categorized as no expression (-), moderate expression characterized by positive nuclear staining in 5- 10% of cells (+), and high expression, characterized by positive nuclear staining in > 10% of cells (++) (Figure 2). As shown in Table 5, only 10% of patients in the nondysplastic SCE group expressed *p53* at a low level, whereas expression was high in the group with high-grade dysplasia (P < 0.01).

DISCUSSION

Several studies, based on endoscopic, biochemical and anthropometric data, have identified GERD, absence of *H. pylori* infection, MS, waist circumference, and body

Table 4 Analysis of variance for the three categories of Barrett's epithelium: non-dysplasia, low-grade dysplasia, and high-grade dysplasia

	No dysplasia (n = 45)	Low grade (n = 14)	High grade (n = 6)	P value
Sex (male/female)	Dec-33	13/1	6/0	0.177
Age (yr)	65.0 ± 9.8	64.3 ± 12.4	63.8 ± 9.4	0.941
Length of BE (SSBE/LSBE)	Feb-43	14/0	4/2	0.044
H. pylori infection rate	Sep-34	6/7	1/5	0.002
p53 positive rate	4/41	7/7	6/0	< 0.001
Body weight (kg)	61.0 ± 9.7	69.5 ± 9.8	65.5 ± 12.5	0.033
BMI (kg/m^2)	23.1 ± 2.8	24.0 ± 3.1	23.3 ± 3.7	0.689
Waist circumference (cm)	86.9 ± 7.9	88.1 ± 9.6	89.3 ± 8.4	0.848
GERD	19/26	9/5	0/6	0.020
Hypertension (BP > 130/85)	29/14	6/7	3/3	0.155
Systolic BP (mmHg)	127 ± 16	120 ± 19	125 ± 6	0.473
Diastolic BP (mmHg)	76 ± 10	69 ± 9	71 ± 6	0.016
Diabetes	14/25	7/6	3/3	0.425
Fasting blood glucose (mg/dL)	118 ± 32	113 ± 19	108 ± 20	0.712
HbA1c (%)	5.6 ± 1.0	5.5 ± 0.7	5.5 ± 0.8	0.971
Dyslipidemia	24/19	5/8	3/3	0.612
TG (mg/dL)	144 ± 58	118 ± 71	183 ± 137	0.190
TC (mg/dL)	202 ± 30	195 ± 36	174 ± 25	0.124
HDL-C (mg/dL)	55 ± 13	55 ± 9	48 ± 7	0.407
LDL-C (mg/dL)	118 ± 26	112 ± 33	97 ± 17	0.201
Fatty liver	17/13	5/7	1/1	0.744
γ-GTP (U/L)	55 ± 48	40 ± 31	101 ± 126	0.097
AST (IU/L)	24 ± 8	26 ± 10	24 ± 10	0.837
ALT (IU/L)	24 ± 15	25 ± 13	21 ± 10	0.803
Hs-CRP (mg/dL)	0.107 ± 0.142	0.104 ± 0.126	0.084 ± 0.029	0.973
Metabolic syndrome	15/14	4/7	2/1	0.604

SSBE: Short segment Barrett esophagus; BMI: Body mass index; LSBE: Long-segment Barrett's esophagus; GERD: Gastroesophageal reflux disease; BP: Blood pressure; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; hs-CRP: High sensitivity C-reactive protein; GTP: Guanosinetriphosphate.

Table 5 Relationship between the level of *p53* expression and grade of dysplasia

	Normal (n = 45)	Low grade (n = 14)	High grade and Ca (n = 6)
p53 (-)	41	7	0
p53 (+)	4	4	4
p53 (++)	0	3	2

Pearson χ^2 test, $P < 0.001$.

weight as risk factors associated with the presence of BE[7-16]. One of the most notable findings from epidemiological reports has been a strong inverse association between *H. pylori* infection and dysplasia of Barrett's epithelium[17,18,21-26].

Esophageal adenocarcinoma derived from BE is not common in Japan as compared with western countries, whereas gastric carcinoma is more prevalent in Japan. This inverse relationship may reflect the high prevalence of *H. pylori* infection in Japan and the low prevalence in western countries[24,39,40].

Another notable epidemiological difference between these regions is the length of BE; that is, SSBE is common in Japan but LSBE is more prevalent in western countries[27,28,30,31]. The underlying reasons for this difference are not currently known.

Here, we identified risk factors associated with the presence of BE dysplasia by comparison of patients with non-dysplasia to those with low- to high-grade dysplasia including adenocarcinoma. In our cohort, 94% of BE cases were the SSBE type (Table 2). Overexpression of *p53* was the most important risk factor associated with dysplasia and adenocarcinoma (Table 3), and the level of *p53* expression was strongly related to the grade of dysplasia (Table 5). Several studies have shown that *p53* overexpression is increased in parallel with progression of histological changes from metaplasia to high-grade dysplasia and adenocarcinoma[41-44]. In specimens obtained from surgical resection, expression of *p53* has been observed in the region of adenocarcinoma as well as in adjacent dysplastic epithelia. In addition, in many cases, *p53* gene mutations are found at the specific position resulting in a change in a specific amino acid residue in both adenocarcinoma and adjacent dysplastic epithelia[41,42]. These results suggest that *p53* mutation, which is relatively uncommon in nondysplastic BE, is an important step in the progression to adenocarcinoma. Galipeau *et al*[45] have shown that inactivation of *p53* by mutation is strongly associated with progression to aneuploidy, possibly through the loss of *p53*-mediated apoptosis and cell-cycle arrest. The accumulation of these aneuploid cell populations has been shown to increase the risk of developing adenocarcinoma[46].

The possible causal role of *p53* in tumorigenesis as well as tumor progression in BE has been postulated, based on histological evidence showing that *p53* mutations are more frequent in advanced stages in histology. Thus, it is important to address the hypothesis that *p53*

overexpression could predict progression of nondysplastic BE to adenocarcinoma. Younes *et al*[47] studied *p53* accumulation *via* immunohistochemistry in 54 patients with Barrett's metaplasia, dysplasia, or adenocarcinoma; *p53* accumulation increased in parallel with histological progression from metaplasia to adenocarcinoma. Follow-up biopsies were available in 23 of 54 patients who had dysplasia in at least one biopsy specimen. They showed that only one of 21 (4.8%) patients who were all *p53* negative in multiple biopsies had histological progression. In contrast, two of three patients with *p53*-positive biopsies progressed to high-grade dysplasia or intramucosal carcinoma (one patient was lost to follow-up). These retrospective data suggest that *p53* accumulation increases the risk of progression from low- to high-grade dysplasia. These data are also consistent with our results showing that the level of *p53* expression was correlated with the grade of dysplasia (Table 5), suggesting that mutated *p53*, which is expressed at an early stage, may stimulate the tumor progression in the metaplasia–dysplasia-adenocarcinoma sequence of BE.

We found a strong inverse association between *H. pylori* infection and dysplasia in BE (Table 2); the lowest prevalence of *H. pylori* was observed in the high-grade dysplasia group (Table 4). Many studies have reported that the absence of *H. pylori* colonization is associated with a greater likelihood of developing esophageal dysplasia and adenocarcinoma[18,20-23]. Hence, *H. pylori* infection appears to have a protective effect against the development of dysplasia and adenocarcinoma in BE. The mechanism through which the absence of *H. pylori* colonization is associated with dysplasia in BE is unknown, but there are several possibilities. First, *H. pylori* infection, in particular the more virulent Cag-A-positive strain, may lead to gastric atrophy resulting in suppression of acid production; this may lower the risk of BE and esophageal adenocarcinoma[24,25].

We analyzed the degree of gastric atrophy according to the Kimura-Takemoto Classification[48] to confirm the association of low acidity and BE dysplasia. We could not find any significant difference in the ratio of atropy and distribution of degree of atrophy in normal individuals and patients with dysplasia (data not shown).

With regard to the possible outcome of *H. pylori* eradication in BE, only a few studies have reported that the SSBE developed at 24 mo after *H. pylori* eradication, suggesting induction of SSBE by the eradication[49]. In Japan, the prevalence of *H. pylori* infection has been decreasing recently and the use of *H. pylori* eradication therapy has flourished. For this reason, the incidence of BE and adenocarcinoma is likely to increase, and it is therefore important to determine risk factors for malignant changes associated with the development of dysplasia and adenocarcinoma in BE after *H. pylori* eradication.

In our multivariate logistic analysis, diastolic blood pressure was an independent risk factor associated with progression of BE from nondysplastic to dysplastic epithelium (Table 3). When we evaluated users of antihypertensive drugs, especially Ca antagonists, in normal individuals and patients with dysplasia, we could not find any significant difference (data not shown). Although this is believed to be the first report of a relationship between diastolic blood pressure and dysplasia in BE, the underlying mechanisms are unclear.

When we analyzed the relationship variables among three categories such as patients with no dysplasia, low- or high- grade dysplasia by ANOVA, we detected no difference in diastolic BP (Table 4). We hypothesized the nonlinear relationship of diastolic BP among the three groups shown in Table 4 as follows: if the variables were associated with malignant potential of Barrett's epithelium, it may show the linear relationship among the three groups, normal, low-grade and high-grade dysplasia. The diastolic BP, however, may be associated with the step such as columnar epithelial metaplasia, which is a relatively early step of transformation.

In our univariate analysis, body weight was extracted as a risk factor for dysplasia in BE, but BMI and waist circumference were not. In a Swedish study of 189 cases of newly diagnosed esophageal adenocarcinoma, a strong positive association was found between BMI and esophageal adenocarcinoma when controlling for GERD symptoms[11]. A study from the Veterans Association in the United States found that individuals with a BMI > 30 had a fourfold greater risk for BE as compared with controls with a BMI < 25[7]. More recently, several studies have revealed that waist circumference, but not BMI, has a modest independent associations with the incidence BE, dysplasia and adenocarcinoma. Other studies have reported that a higher waist-to-hip ratio is associated with BE when data are adjusted for GERD symptoms and BMI[14,50]. In our multivariate logistic analysis, anthropometric variables were not extracted as risk factors.

In conclusion we demonstrated that *p53* overexpression, absence of *H. pylori* infection, and low diastolic BP are independent risk factors associated with the dysplastic changes of BE. Future studies such as well-designed prospective studies are needed to elucidate the mechanisms underlying the association of these risk factors with the sequence of progression of dysplasia to adenocarcinoma in BE and establish a high-rsk group progressing to adenocarcinoma.

COMMENTS

Background

Barrett's esophagus (BE) is defined as a condition in which normal squamous mucosa is replaced by columnar epithelium. This intestinal metaplasia of the distal esophagus is considered to be a premalignant condition where metaplasia may progress to dysplasia and subsequently to adenocarcinoma. It has been reported that central adiposity, metabolic syndrome, and high body mass index (BMI) are associated with BE and adenocarcinoma.

Research frontiers

In order to identify variables associated with BE dysplasia, many variables taken from biochemical and anthropometric data, *p53* expression examined by immunostaining and *Helicobacter pylori* (*H. pylori*) infection were analyzed. Consequently, the authors analyzed many different types of variables.

Innovations and breakthroughs

Only three variables were selected by multivariate analysis. *p53* stainig was positively associated with dysplasia, but *H. pylori* infection and diastolic blood pressure were inversely associated with BE dysplasia.

Applications

Using these three variables, a prospective study to determine the high-risk group for BE dysplasia or factors related to progression of dysplasia may be the next step.

Terminology

BE is defined as intestinal metaplasia in which normal squamous mucosa is replaced by columnar epithelium. BE is characterized by three types of columnar epithelium, namely, cardiac type (junctional type), fundic type, and intestinal metaplasia type [specialized columnar epithelium type (SCE) type]. In these three type of BE, SCE type is especially considered to be a precancerous lesion of esophageal adenocarcinoma.

Peer review

The authors showed the risk factors associated with Barrett's epithelial dysplasia. They enrolled 151 patients with BE in a single hospital. This study was well organized and well investigated. They clearly showed that *p53* expression, absence of *H. pylori* infection and low diastolic blood pressure are risk factors associated with dysplasia of BE.

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