ORIGINAL ARTICLE

Gut and Liver, Vol. 8, No. 3, May 2014, pp. 277-281

Low Pepsinogen I Level Predicts Multiple Gastric Epithelial Neoplasias for Endoscopic Resection

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Background/Aims: Synchronous/metachronous gastric epithelial neoplasias (GENs) in the remaining lesion can develop at sites other than the site of endoscopic resection. In the present study, we aimed to investigate the predictive value of serum pepsinogen for detecting multiple GENs in patients who underwent endoscopic resection. **Methods:** In total, 228 patients with GEN who underwent endoscopic resection and blood collection for pepsinogen I and II determination were evaluated retrospectively. **Results:** The mean period of endoscopic follow-up was 748.8±34.7 days. Synchronous GENs developed in 46 of 228 (20.1%) and metachronous GENs in 27 of 228 (10.6%) patients during the follow-up period. Multiple GENs were associated with the presence of pepsinogen I <30 ng/mL (p<0.001). Synchronous GENs were associated with the presence of pepsinogen I <30 ng/mL (p<0.001). **Conclusions:** Low pepsinogen I levels predict multiple GENs after endoscopic resection, especially synchronous GENs. Cautious endoscopic examination prior to endoscopic resection to detect multiple GENs should be performed for these patients. **(Gut Liver 2014;8:277-281)**

Key Words: Pepsinogens; Stomach; Neoplasms

INTRODUCTION

Endoscopic resection (endoscopic mucosal resection [EMR] or endoscopic submucosal dissection [ESD]) has been accepted as a curative modality for gastric epithelial neoplasia (GEN) including early gastric cancer.[1,2] Compared with surgery, endoscopic resection is considered a minimal invasive technique capable of preserving large quantity of gastric mucosa. However, synchronous/metachronous GENs in the remaining lesion could develop at sites other than that of endoscopic resection. There have been several reports on the occurrence of metachronous cancer after endoscopic treatment.[3-6] During the follow-up after endoscopic resection, metachronous tumor was found to develop at rate of 1% to 3% per year.[7] Therefore, characterization of GEN with high risk for synchronous/metachronous lesion is required for follow-up and treatment of patients, who underwent endoscopic resection of GEN.

Human pepsinogen I and II are proenzymes of pepsin, an endoproteinase of gastric juice. Pepsinogen I is secreted mainly by chief cells in fundic mucosa,[8] whereas pepsinogen II is secreted by pyloric glands and proximal duodenal mucosa.[9] *Helicobacter pylori*-related gastric atrophy and further changes tend to begin at the antrum and progress proximally toward corpus. Serum pepsinogen I and II concentrations and the ratio between pepsinogen I/II may be related to histologic and functional status of gastric mucosa.[10] In addition, a stepwise course of *H. pylori* infection has been defined as a sequence of histological events that confer an increasing risk of malignant formation, as described in Correa hypothesis. In this process, intestinal metaplasia is believed to be the precancerous lesion of the stomach.[11,12]

In the present study, we aimed to investigate the predictive value of serum pepsinogen for detection of multiple GENs in patients who underwent endoscopic resection.

MATERIALS AND METHODS

1. Study participants

The patients who hospitalized from March 2008 to March 2011 were enrolled. Patients with medical history of endoscopic resection for GEN were eligible for participation. Exclusion criteria were a history of gastrectomy or *H. pylori* eradication,

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Received on April 29, 2013. Revised on June 6, 2013. Accepted on June 26, 2013. Published online on January 14, 2014
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl.2014.8.3.277

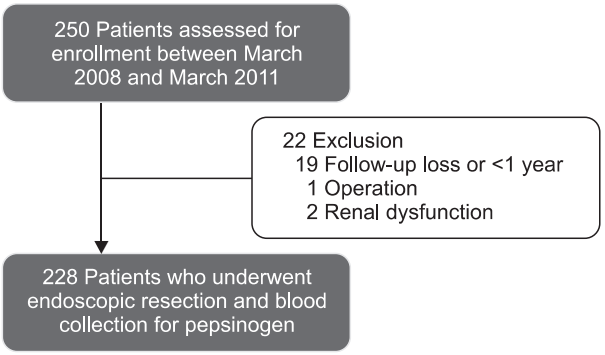

Fig. 1. Flow sheet of enrolled patients. Of the 250 patients with gastric epithelial neoplasia who were initially treated with endoscopic resection, we excluded 22 patients, one of whom underwent gastric surgery and two of whom had renal dysfunction. Consequently, 228 patients were enrolled in this study.

history of medication such as antacid or proton-pump inhibitor, incomplete resection and advanced cancer or beyond submucosal invasion requiring subsequent surgery. Total of 250 patients with GEN who underwent EMR or ESD and blood collections for pepsinogen I and II determination were enrolled in this study. We excluded 19 patients whose surveillance periods less than 1 year, one patient who underwent gastric surgery within 1 year, and two patients who had renal dysfunction. Consequently, 228 patients who underwent endoscopic resection and blood collections for pepsinogen I and II determination were evaluated retrospectively (Fig. 1). This study was approved by the Institutional Review Board of Chonnam National University Hospital.

2. Definition of nomenclature: synchronous and metachronous GEN

Synchronous GEN is defined as multiple tumors at diagnosis or a newly detected tumor within 1 year after the initial resection. Metachronous GEN is defined as a newly-developed tumor after 1 year of endoscopic resection. We defined multiple GENs as synchronous and metachronous GENs.

3. Serum sample

Fasting serum samples were collected from the patients and serum concentrations of pepsinogen I and II were measured using a latex-enhanced Turbidimetric Immunoassay (Shima Laboratories, Tokyo, Japan). No other acid suppressants were used before the blood sampling. Absence of atrophy was defined as both pepsinogen I >70 and pepsinogen I/II ratio >3.0. Severe atrophy was defined as pepsinogen ≤30 and pepsinogen I/II ≤2.0.

4. Endoscopic procedure and *H. pylori* status

EMR or ESD procedure using upper gastrointestinal endoscopy (GIF H 260; Olympus, Tokyo, Japan) was performed. Tumor location (long axis) was classified by dividing the stomach into three equal sections-upper (cardia, fundus, upper body), middle (mid body, lower body, angle), and lower (antrum, prepylorus).

Patients were considered to be infected with *H. pylori* if at least one of three test results (rapid urease test, histology results, or [^{13}C]-urea breath test) was positive.

5. Follow-up endoscopy

Two months after EMR or ESD, gastroscopy was performed to exclude the presence of synchronous multiple GENs. After this, surveillance endoscopy was scheduled once or twice a year to diagnose metachronous GEN. Endoscopic biopsies were performed at the EMR or ESD site at every follow-up to exclude local recurrence. Additional endoscopic biopsies were performed if any other gastric neoplasm was suspected.

6. Clinicopathologic evaluation for GEN

The maximum tumor diameter of GEN was measured macroscopically as the tumor size. GENs were divided into three gross types: protruding, superficial elevated, and superficial depressed. All resected specimens were evaluated by histologic examination on the basis of the Vienna classification.[13]

7. Statistical analysis

Differences in categorical variables between groups were analyzed using the chi-square test or Fisher exact test when required. Differences in continuous variables between groups were analyzed using the t-test or Mann-Whitney test. Predictive factors with p-values <0.05 as determined by univariate analysis were included in multivariate analysis by logistic regression. Odd ratios and 95% confidence intervals were calculated for each variable for multivariate analysis. The follow-up period was measured from the date of endoscopic resection to the detection date of last endoscopic examination. p-values <0.05 were considered significant. All data analysis was conducted with SPSS version 20.0 for Windows (IBM Co., Armonk, NY, USA).

RESULTS

1. Baseline characteristics of patients with gastric epithelial neoplasm

Table 1 shows the baseline characteristics of 228 patients enrolled in this study. The mean age of patients was 64.4 years. Male patients comprised 70.6% of enrolled patients. The mean follow-up period after endoscopic resection was 748.8±34.7 days. During follow-up period after endoscopic resection, only 38 patients among *H. pylori*-positive patients (38/70, 54.3%) received eradication therapy. During the follow-up period, 70 (30.7%) patients had multiple GENs. Forty-six patients (20.1%) had synchronous GENs within 1 year of endoscopic resection. Among these patients, 37 patients (16.2%) had synchronous GENs at initial endoscopic resection. Twenty-four patients (10.6%) developed metachronous GENs after 1 year of endoscopic resection. Among 70 patients with multiple GENs, there

Table 1. Baseline Characteristics of Patients with Gastric Epithelial Neoplasm

Characteristic	Total	Single	Synchronous	Metachronous	p-value
Incidence	228 (100)	158 (69.3)	46 (20.1)	24 (10.6)	
Mean age, yr	64.4±8.8	63.9±9.5	66.1±7.1	64.4±6.4	0.311
Sex (male/female)	161/67	111/47	33/13	17/7	0.981
Macroscopic type					0.083
Protruding	11 (4.8)	6 (3.8)	4 (8.7)	1 (4.2)	
Flat elevated	163 (71.5)	106 (67.1)	40 (87.0)	17 (70.8)	
Flat	12 (5.2)	11 (7.0)	0 (0.0)	1 (4.2)	
Flat depressed	42 (18.5)	35 (22.1)	2 (4.3)	5 (18.5)	
Mean tumor size (SEM), mm	18.6±0.6	18.4±0.7	19.3±1.3	18.8±2.3	0.846
Location					0.140
Upper 1/3	8 (3.5)	5 (3.2)	0 (0.0)	3 (12.5)	
Middle 1/3	80 (35.1)	55 (34.8)	14 (30.4)	11 (45.8)	
Lower 1/3	140 (61.4)	98 (62.0)	32 (69.6)	10 (41.7)	
Histological type					0.531
Low grade dysplasia	82 (36.0)	57 (36.1)	18 (39.1)	7 (29.2)	
High grade dysplasia	71 (31.1)	46 (29.1)	18 (39.1)	7 (29.2)	
Differentiated EGC	71 (31.1)	52 (32.9)	9 (19.6)	10 (41.7)	
Undifferentiated EGC	4 (1.8)	3 (1.9)	1 (2.2)	0 (0.0)	
Presence of *H. pylori*	70 (42.1)	50 (31.2)	9 (19.6)	11 (45.8)	0.070
Mean pepsinogen, ng/mL	50.4±40.5	56.7±40.5	25.0±23.4	57.2±31.6	<0.001*
T†		a	b	a	
Pepsinogen I <30 ng/mL	81 (35.5)	42 (25.6)	33 (71.7)	6 (25.0)	<0.001
Mean pepsinogen I/II	3.4±2.4	3.7±2.6	2.4±1.7	3.2±1.8	0.008*
T†		a	b	a	
Pepsinogen I/II <2.0	73 (32.2)	43 (27.4)	24 (52.2)	6 (25.0)	0.005

Data are presented as number (%), mean±SD, or mean±SEM.
SEM, standard error of the mean; EGC, early gastric cancer; *H. pylori, Helicobacter pylori.*
*Statistical significances were tested by one-way analysis of variances among groups; †The same letters (a,b) indicated nonsignificant difference between the groups based on Tukey's multiple comparison test.

Table 2. Univariate Logistic Regression Analysis for the Prediction Associated with the Presence of Multiple Gastric Epithelial Neoplasias (GENs), Synchronous GENs, and Metachronous GENs

	Single vs Multiple GENs		Single vs Synchronous GENs		Single vs Metachronous GENs	
	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Pepsinogen I <30 ng/mL	3.56 (1.97-6.43)	<0.001	7.01 (3.37-14.6)	<0.001	1.09 (0.40-2.92)	0.870
Pepsinogen I/II <2.0	1.98 (1.10-3.58)	0.020	2.89 (1.47-5.69)	0.002	1.13 (0.42-3.04)	0.806

Multiple GENs means synchronous GENs or metachronous GENs.
aOR, adjusted odds ratio; CI, confidence interval.

were low grade dysplasia in 43 patients, high grade dysplasia in 16 patients, well differentiated adenocarcinoma in 10 patients, and poorly differentiated adenocarcinoma in one patient. Only two patients underwent surgery for multiple GENs. The remainder underwent endoscopic resection for multiple GENs.

2. Comparison between patients with single GEN and patients with multiple GENs

There was no significant difference in age, sex, the presence of *H. pylori*, and tumor characteristics between the two groups. However, pepsinogen I <30 (p<0.001) and pepsinogen I/II <2.0 (p=0.020) were significantly associated with the presence of

Table 3. Multivariate Logistic Regression Analysis for Predicting the Association with the Presence of Multiple Gastric Epithelial Neoplasias (GENs), Synchronous GENs, and Metachronous GENs

	Single vs Multiple GENs		Single vs Synchronous GENs		Single vs Metachronous GENs	
	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Pepsinogen I <30 ng/mL	3.45 (1.59-7.45)	0.002	6.99 (2.71-17.9)	<0.001	1.01 (0.28-3.60)	0.990
Pepsinogen I/II <2.0	1.13 (0.52-2.48)	0.762	1.09 (0.43-2.73)	0.860	1.16 (0.33-4.11)	0.818

Multiple GENs means synchronous GENs or metachronous GENs.
aOR, adjusted odds ratio; CI, confidence interval.

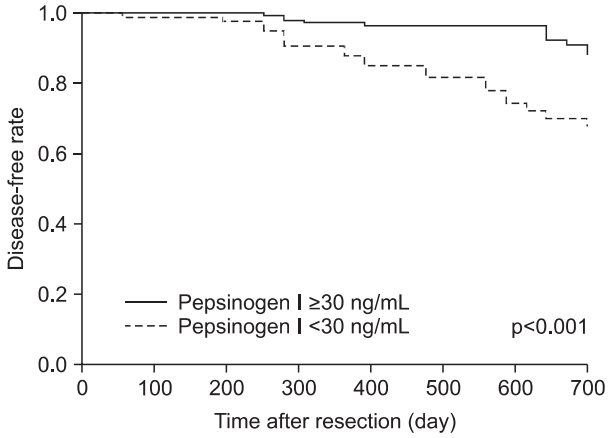

Fig. 2. Kaplan-Meier curve for gastric epithelial neoplasia (GEN) patients with synchronous or metachronous GENs after initial endoscopic resection. Overall disease-free curve in relation to pepsinogen I levels in patients with GENs. There was a significant difference in the 2-year disease-free rate between the pepsinogen I ≥30 ng/mL and pepsinogen I <30 ng/mL groups (p<0.001).

multiple GENs (Table 2).

3. Independent predictive factor of multiple GENs

Two risk factors with p-values <0.05 as determined by univariate analysis (pepsinogen I <30 and pepsinogen I/II <2.0), age and *H. pylori* positivity were included in the multivariate logistic regression analysis, and significant risk factors were selected by the stepwise method. Multiple GENs were more associated with the presence of pepsinogen I <30 ng/mL (p<0.001). Synchronous GENs were more associated with the presence of pepsinogen I <30 ng/mL (p<0.001) (Table 3). Fig. 2 showed 2-year disease free rates by subclassification with pepsinogen I level.

DISCUSSION

The pepsinogen I test and the ratio of pepsinogen I/II have been used in the diagnosis of atrophic corpus gastritis worldwide for decades. In case-control study from Japan, the highest cancer risk (risk ratio, 25) was observed in patients with low plasma levels of pepsinogen I or with a low ratio of pepsinogen I/II. All these patients exhibited multifocal atrophic gastritis (atrophic gastritis in both the antrum and corpus) in endoscopy and biopsy histology.[14] In fact, pepsinogen test has been used as a noninvasive serology biopsy to select and improve patient compliance to the generalized cancer screening program in Japan.[15,16]

Once gastric cancer has developed in the stomach for the first time, the background mucosa has higher potential of having another gastric cancer. Moreover, after endoscopic resection of tumor and surrounding mucosa, the possibility of synchronous gastric cancer still remains and metachronous gastric cancer could develop at sites other than that of endoscopic resection. Since, atrophic gastritis with or without intestinal metaplasia already exists in the background mucosa and is regarded as a premalignant lesion; this background mucosa may be regarded as risk factor for GENs such as low grade dysplasia or high grade dysplasia. The present study showed that the incidence of multiple GENs was 30.7% and the incidence of metachronous lesions was 10.6%, which were similar to those of previous studies (5.6% to 14.6%).[2-4,8,17] Generally, during follow-up after endoscopic resection, metachronous tumor develops at a rate of 1% to 3% per year.[7]

Both the endoscopist and the patient undergoing endoscopic resection of GEN will be embarrassed if another lesion was detected within a short follow-up period. Therefore, it is necessary to clarify which clinicopathologic characteristics are predictive markers for synchronous or metachronous GENs before performing endoscopic resection. For early detection and curative treatment of synchronous or metachronous GENs, periodic and meticulous endoscopic examinations of the gastric remnants are very important. Also, it would be beneficial to develop a stratified endoscopic surveillance program. Previous reports have shown that microsatellite instability may play an important role in the development of synchronous or metachronous gastric cancer and that it could be used clinically as a molecular marker for the prediction of multiple gastric cancers.[18,19] Another study showed that the risk of metachronous cancer after *H. pylori* eradication correlated with the severity of corpus atrophy.[17] Han *et al.*[20] reported that endoscopically assessed antral atrophy and old age were significantly associated with the incidence of metachronous gastric cancer after endoscopic resection. The current study showed that pepsinogen I <30 ng/mL (adjusted odds ratio, 3.33) was an independent risk factor for multiple GENs (especially, synchronous GENs). For these reasons, endos-

copists should make an effort to detect any synchronous lesions around the time of endoscopic resection and implement an intensive follow-up program in patients with low pepsinogen level in a short period time. Fukase *et al.*[6] reported that prophylactic eradication of *H. pylori* after endoscopic resection of early gastric cancer should be used to prevent the development of metachronous gastric carcinoma.[3] In the present study, the rate of *H. pylori* infection was only 42%. However, we could not show the past infection status (such as immunoglobulin assay or enzyme-linked immunosorbent assay) and effect of eradication of *H. pylori.* Actually, the current study could not show the relationship of low pepsinogen I and metachronous lesion. After endoscopic resection of GENs, many patients make an effort to modify their life style modification (low salt diet, high fiber diet, quit smoking) and take a medication such as *H. pylori* eradication. These factors may improve the inflammatory status of gastric mucosa. However, current study did not reflect these factors. Besides the above mentioned findings, the following limitation should be considered when interpreting the presented results. We analyzed the retrospective data for the relative small number of enrolled patients, leading to the possibility of selection bias. Therefore, further large-scale studies are required to confirm the indentified important risk factors associated with synchronous or metachronous GENs.

In conclusion, low pepsinogen I and open type gastric atrophy predict multiple GENs regarding endoscopic resection. Cautious endoscopic examination before endoscopic resection to detect multiple GENs should be performed for these patients regarding endoscopic resection.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Nasu J, Doi T, Endo H, Nishina T, Hirasaki S, Hyodo I. Characteristics of metachronous multiple early gastric cancers after endoscopic mucosal resection. Endoscopy 2005;37:990-993.
2. Arima N, Adachi K, Katsube T, et al. Predictive factors for metachronous recurrence of early gastric cancer after endoscopic treatment. J Clin Gastroenterol 1999;29:44-47.
3. Uemura N, Mukai T, Okamoto S, et al. Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. Cancer Epidemiol Biomarkers Prev 1997;6:639-642.
4. Kikuchi S, Kato M, Katsuyama T, Tominaga S, Asaka M. Design and planned analyses of an ongoing randomized trial assessing the preventive effect of Helicobacter pylori eradication on occurrence of new gastric carcinomas after endoscopic resection. Helicobacter 2006;11:147-151.
5. Kato M, Asaka M, Shimizu Y, et al. Relationship between Helicobacter pylori infection and the prevalence, site and histological type of gastric cancer. Aliment Pharmacol Ther 2004;20 Suppl 1:85-89.
6. Fukase K, Kato M, Kikuchi S, et al. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet 2008;372:392-397.
7. Kim SG. Endoscopic treatment for early gastric cancer. J Gastric Cancer 2011;11:146-154.
8. Samloff IM. Cellular localization of group I pepsinogens in human gastric mucosa by immunofluorescence. Gastroenterology 1971;61:185-188.
9. Samloff IM, Liebman WM. Cellular localization of the group II pepsinogens in human stomach and duodenum by immunofluorescence. Gastroenterology 1973;65:36-42.
10. Varis K, Sipponen P, Laxén F, et al. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia: Helsinki Gastritis Study Group. Scand J Gastroenterol 2000;35:950-956.
11. Correa P. A human model of gastric carcinogenesis. Cancer Res 1988;48:3554-3560.
12. Filipe MI, Muñoz N, Matko I, et al. Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. Int J Cancer 1994;57:324-329.
13. Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. Gut 2000;47:251-255.
14. Kikuchi R, Abe Y, Iijima K, et al. Low serum levels of pepsinogen and gastrin 17 are predictive of extensive gastric atrophy with high-risk of early gastric cancer. Tohoku J Exp Med 2011;223:35-44.
15. Fahey MT, Hamada GS, Nishimoto IN, et al. Ethnic differences in serum pepsinogen levels among Japanese and non-Japanese Brazilian gastric cancer patients and controls. Cancer Detect Prev 2000;24:564-571.
16. Kato I, Miki K, Muñoz N, et al. Determinants of plasma pepsinogen levels in a population at high risk for stomach cancer in Venezuela. Int J Cancer 1995;62:512-518.
17. Shiotani A, Uedo N, Iishi H, et al. Predictive factors for metachronous gastric cancer in high-risk patients after successful Helicobacter pylori eradication. Digestion 2008;78:113-119.
18. Miyoshi E, Haruma K, Hiyama T, et al. Microsatellite instability is a genetic marker for the development of multiple gastric cancers. Int J Cancer 2001;95:350-353.
19. Hasuo T, Semba S, Li D, et al. Assessment of microsatellite instability status for the prediction of metachronous recurrence after initial endoscopic submucosal dissection for early gastric cancer. Br J Cancer 2007;96:89-94.
20. Hanaoka N, Uedo N, Shiotani A, et al. Autofluorescence imaging for predicting development of metachronous gastric cancer after Helicobacter pylori eradication. J Gastroenterol Hepatol 2010;25:1844-1849.