Table 5 No. glands with gastric and intestinal mixed endocrine cells/total glands

	F	Pseudo-P	P	GI-IM	I-IM-Pa(–)	I-IM-Pa(+)	Total
Fundic mucosa	1/214 (0.5%)*	1/98 (1.0%)*	–	44/265 (16.6%)	3/145 (2.1%)*	4/308 (1.3%)*	53/1030 (5.1%)
Pyloric mucosa	–	–	23/569 (4.0%)*	306/1290 (23.7%)	7/40 (17.5%)	11/105 (10.5%)**,***	347/2004 (17.3%)

F, fundic glandular duct; GI-IM, gastric and intestinal mixed phenotype IM; I-IM-Pa(–), solely intestinal phenotype IM without Paneth cell; I-IM-Pa(+), solely intestinal phenotype IM with Paneth cell; IM, intestinal metaplasia; P, pyloric glandular duct; pseudo-P, pseudo pyloric glandular duct.
*$P < 0.0001$, compared with GI-IM. **$P < 0.01$, compared with GI-IM. ***$P = 0.0012$, compared with P.

markers such as MUC5AC, MUC6, MUC2, and villin. Jenny *et al.* have previously demonstrated that neurogenin3 is required for endocrine cell fate specification in multipotent intestinal progenitor cells, whereas gastric endocrine development is both neurogrnin3 dependent and independent.[33] In addition, we consider that Cdx2 might be important in the regulation of the intestinal endocrine cell markers such as glicentin, GIP, and GLP-1, because its expression can be detected at the bottom of small intestinal and colonic glandular ducts. Recently, La Rosa *et al.* demonstrated that Cdx2 may be a sensitive and specific marker of midgut endocrine cells and endocrine tumors.[34] Thus specific transcription factors, including Cdx2, might play an important role in the intestinalization of both mucous and endocrine cells.

In pyloric glandular ducts with chronic gastritis, expression of the intestinal endocrine cell marker was low but present, in contrast to the normal pyloric mucosa without inflammation. With regard to the mucous cell markers, we earlier demonstrated the mRNA expression of *Cdx2* and *villin* in glandular ducts that are regarded as typical of G-type glands in the stomach of chronic gastritis.[29] Eda *et al.* have previously suggested that Cdx2 triggers the initiation and development of IM in chronic gastritis, from analysis of mRNA levels.[35] Satoh *et al.* described the expression of this in gastric epithelium of *H. pylori*-infected patients without IM.[36] Ishihara *et al.* proposed that *H. pylori* infection is a factor inducing glicentin in the gastric mucosa.[37] We therefore consider that change in transcriptional elements responsible for intestinal phenotypic expression of both mucous and endocrine cells might be the initial event in intestinalization of gastric mucosa in cases of chronic gastritis.

We have here demonstrated that the phenotype of endocrine cells in pseudopyloric glandular ducts is similar to those in the normal pyloric glandular ducts rather than those in the fundic glands. Furthermore, no gastrin-positive endocrine cells have been found in I-IM including both I-IM-Pa(–) and I-IM-Pa(+), resembling normal fundic glands. Thus, we consider the hypothesis that GI-IM in fundic mucosa could be preceded by pseudopyloric metaplasia with a similar pathogenesis occurring in pyloric glands, whereas some of the I-IM developing in fundic glands might be emerging directly from normal fundic glands without passing through pseudopyloric metaplasia. *Pancreatic–duodenal homeobox 1* (*Pdx1*) is a ParaHox gene, which contributes to genesis and development of pancreas, duodenum, and antrum. Pdx1 plays an important role in the development of pseudopyloric glands.[38] Thus, we consider that it is very important to clarify the regulation of mucous and endocrine cell markers from the viewpoint of transcriptional elements, discriminating pseudo-P glands from fundic mucosa in the human stomach. In the light of the clonal findings with C3H/HeN⇔BALB/c chimeric mice, we consider that the alteration from fundic to pseudopyloric glands must be derived at the stem cell level.

In conclusion, our results suggest that the phenotypes of endocrine cells might be determined in tandem with those of their mucous counterparts in stomach glands. The alternation of phenotypes in mucous cells also involves those in endocrine cells and our results provide support for the concept that all of the different types of mucous and endocrine cells in normal and intestinal metaplastic glands might be derived from a single progenitor cell in each gland.

ACKNOWLEDGMENTS

The authors thank Dr Malcolm A. Moore for revision of the scientific English language, and Ms Hisayo Ban for expert technical assistance. The present study was supported in part by a Grant-in-Aid for the Third-term Comprehensive 10 year Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1 Byrd JC, Yan P, Sternberg L, Yunker CK, Scheiman JM, Bresalier RS. Aberrant expression of gland-type gastric mucin in the surface epithelium of *Helicobacter pylori*-infected patients. *Gastroenterology* 1997; **113**: 455–64.
2 Dixon MF. Histological responses to *Helicobacter pylori* infection: gastritis, atrophy and preneoplasia. *Baillieres Clin Gastroenterol* 1995; **9**: 467–86.
3 Morson BC. Intestinal metaplasia of the gastric mucosa. *Br J Cancer* 1955; **9**: 365–76.
4 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735–40.
5 You WC, Blot WJ, Li JY *et al.* Precancerous gastric lesions in a population at high risk of stomach cancer. *Cancer Res* 1993; **53**: 1317–21.
6 Stemmermann GN. Intestinal metaplasia of the stomach. A status report. *Cancer* 1994; **74**: 556–64.
7 Filipe MI, Munoz N, Matko I *et al.* Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. *Int J Cancer* 1994; **57**: 324–9.
8 Kawachi T, Kogure K, Tanaka N, Tokunaga A, Sugimura T. Studies of intestinal metaplasia in the gastric mucosa by detection of disaccharidases with 'Tes-Tape'. *J Natl Cancer Inst* 1974; **53**: 19–30.
9 Jass JR, Filipe MI. A variant of intestinal metaplasia associated with gastric carcinoma: a histochemical study. *Histopathology* 1979; **3**: 191–9.
10 Tatematsu M, Tsukamoto T, Inada K. Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. *Cancer Sci* 2003; **94**: 135–41.
11 Inada K, Nakanishi H, Fujimitsu Y *et al.* Gastric and intestinal mixed and solely intestinal types of intestinal metaplasia in the human stomach. *Pathol Int* 1997; **47**: 831–41.
12 Tsukamoto T, Inada K, Tanaka H *et al.* Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. *J Cancer Res Clin Oncol* 2004; **130**: 135–45.
13 Yuasa H, Inada K, Watanabe H, Tatematsu M. A phenotypic shift from gastric-intestinal to solely intestinal cell types in intestinal metaplasia in rat stomach following treatment with X-rays. *J Toxicol Pathol* 2002; **15**: 85–93.
14 Nozaki K, Shimizu N, Tsukamoto T *et al.* Reversibility of heterotopic proliferative glands in glandular stomach of helicobacter pylori-infected mongolian gerbils on eradication. *Jpn J Cancer Res* 2002; **93**: 374–81.
15 Sjolund K, Sanden G, Hakanson R, Sundler F. Endocrine cells in human intestine: an immunocytochemical study. *Gastroenterology* 1983; **85**: 1120–30.
16 Owen DA. Normal histology of the stomach. *Am J Surg Pathol* 1986; **10**: 48–61.
17 Hocker M, Wiedenmann B. Molecular mechanisms of enteroendocrine differentiation. *Ann NY Acad Sci* 1998; **859**: 160–74.
18 Oberg K. Gastric neuroendocrine cells and secretory products. *Yale J Biol Med* 1998; **71**: 149–54.
19 Thim L, Moody AJ. The primary structure of porcine glicentin (proglucagon). *Regul Pept* 1981; **2**: 139–50.
20 Tsutsumi Y, Nagura H, Watanabe K, Yanaihara N. A novel subtyping of intestinal metaplasia of the stomach, with special reference to the histochemical characterizations of endocrine cells. *Virchows Arch A Pathol Anat Histopathol* 1983; **401**: 73–88.
21 Meier JJ, Nauck MA, Schmidt WE, Gallwitz B. Gastric inhibitory polypeptide: the neglected incretin revisited. *Regul Pept* 2002; **107**: 1–13.
22 Schirra J, Wank U, Arnold R, Goke B, Katschinski M. Effects of glucagon-like peptide-1(7-36)amide on motility and sensation of the proximal stomach in humans. *Gut* 2002; **50**: 341–8.
23 Bordi C, Ravazzola M. Endocrine cells in the intestinal metaplasia of gastric mucosa. *Am J Pathol* 1979; **96**: 391–8.
24 Mingazzini P, Carlei F, Malchiodi-Albedi F *et al.* Endocrine cells in intestinal metaplasia of the stomach. *J Pathol* 1984; **144**: 171–8.
25 Katsuyama T, Spicer SS. Histochemical differentiation of complex carbohydrates with variants of the concanavalin A-horseradish peroxidase method. *J Histochem Cytochem* 1978; **26**: 233–50.
26 Katsuyama T, Ono K, Nakayama J, Akamatsu T, Honda T. Mucosubstance histochemistry of the normal mucosa and carcinoma of the large intestine. Galactose oxidase-Schiff reaction and lectin stainings. *Acta Pathol Jpn* 1985; **35**: 1409–25.
27 Mizoshita T, Tsukamoto T, Inada K *et al.* Immunohistochemically detectable Cdx2 is present in intestinal phenotypic elements in early gastric cancers of both differentiated and undifferentiated types, with no correlation to non-neoplastic surrounding mucosa. *Pathol Int* 2004; **54**: 392–400.
28 Mizoshita T, Tsukamoto T, Nakanishi H *et al.* Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. *J Cancer Res Clin Oncol* 2003; **129**: 727–34.
29 Tsukamoto T, Inada K, Tanaka H *et al.* Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. *J Cancer Res Clin Oncol* 2004; **130**: 135–45.

30 Tatematsu M, Fukami H, Yamamoto M *et al.* Clonal analysis of glandular stomach carcinogenesis in C3H/HeN<==>BALB/c chimeric mice treated with N-methyl-N-nitrosourea. *Cancer Lett* 1994; **83**: 37–42.

31 Tatematsu M, Masui T, Fukami H *et al.* Primary monoclonal and secondary polyclonal growth of colon neoplastic lesions in C3H/HeN<==>BALB/c chimeric mice treated with 1,2-dimethylhydrazine immunohistochemical detection of C3H strain-specific antigen and simple sequence length polymorphism analysis of DNA. *Int J Cancer* 1996; **66**: 234–8.

32 Ito H, Yokozaki H, Hata J, Mandai K, Tahara E. Glicentin-containing cells in intestinal metaplasia, adenoma and carcinoma of the stomach. *Virchows Arch A Pathol Anat Histopathol* 1984; **404**: 17–29.

33 Jenny M, Uhl C, Roche C *et al.* Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *EMBO J* 2002; **21**: 6338–47.

34 La Rosa S, Rigoli E, Uccella S, Chiaravalli AM, Capella C. CDX2 as a marker of intestinal EC-cells and related well-differentiated endocrine tumors. *Virchows Arch* 2004; **445**: 248–54.

35 Eda A, Osawa H, Yanaka I *et al.* Expression of homeobox gene CDX2 precedes that of CDX1 during the progression of intestinal metaplasia. *J Gastroenterol* 2002; **37**: 94–100.

36 Satoh K, Mutoh H, Eda A *et al.* Aberrant expression of CDX2 in the gastric mucosa with and without intestinal metaplasia: effect of eradication of Helicobacter pylori. *Helicobacter* 2002; **7**: 192–8.

37 Ishihara S, Fukuda R, Moriyama N *et al.* Helicobacter pylori infection accelerates gene expression of glicentin in the gastric mucosa. Its association with intestinal metaplasia of the stomach. *Scand J Gastroenterol* 1997; **32**: 460–64.

38 Sakai H, Eishi Y, Li XL *et al.* PDX1 homeobox protein expression in pseudopyloric glands and gastric carcinomas. *Gut* 2004; **53**: 323–30.

RESEARCH COMMUNICATION

Suppressive Effects of Fruit-juice Concentrate of Prunus Mume Sieb. et Zucc. (Japanese apricot, Ume) on Helicobacter Pylori-induced Glandular Stomach Lesions in Mongolian Gerbils

Takafumi Otsuka[1,2], Tetsuya Tsukamoto[1], Harunari Tanaka[1], Kenichi Inada[1], Hirotoshi Utsunomiya[3], Tsutomu Mizoshita[1], Toshiko Kumagai[4], Tsutomu Katsuyama[4], Kazumasa Miki[2], Masae Tatematsu[1]

Abstract

***Helicobacter pylori* (*Hp*) infection is an important factor in human gastric disorders, including chronic active gastritis, peptic ulcers, intestinal metaplasia and cancer. Since epidemiologic studies overwhelmingly agree on a protective influence of fruits and vegetables in reducing the risk of gastric neoplasia and processed foods made from Prunus mume Sieb. et Zucc. (Japanese apricot or "Ume" in Japanese) are traditionally known for their miscellaneous medical effects, in the present study we investigated the efficacy of a fruit-juice concentrate of Japanese apricot (CJA) in the glandular stomach of *Hp*-infected Mongolian gerbils. *Hp*-inoculated gerbils were given CJA in their drinking water at concentrations of 1 and 3% for 10 weeks. The microscopic scores for gastritis and mucosal hyperplasia in the CJA groups were significantly lower than in the *Hp*-inoculated control group, with dose-dependence. Real-time PCR was performed to quantitate *Hp* by demonstrating urease A gene amount using gerbils' glyceraldehyde-3-phophate dehydrogenase (GAPDH) gene as an internal control. Average relative urease A gene dosage in the glandular stomach in the 1 and 3% CJA and *Hp*-inoculated control groups was 26.6 ± 11.6% (average ± SE), 30.3 ± 10.5%, 100 ± 40.9%, respectively, the fruit-juice concentrate causing significant lowering (P<0.01 and P<0.05, respectively, with 1 and 3%). These findings suggest that suppressive effects on gastric cancer development might also be expected as a result of decreased numbers of *Hp* and improvement of *Hp*-induced chronic active gastritis on administration of CJA.**

Key Words: Helicobacter pylori - Mongolian gerbils - Prunus mume Sieb. et Zucc. (Japanese apricot, Ume) - glandular stomach - inflammation

Asian Pacific J Cancer Prev, 6, 337-341

Introduction

Helicobacter pylori (*Hp*) is a major causative factor for gastric disorders and epidemiological evidence has accumulated indicating a significant relationship with chronic active gastritis, peptic ulcer, atrophic gastritis, intestinal metaplasia, and lymphoma or cancer development (Marshall and Warren, 1984; Nomura et al., 1991; Uemura et al., 2001). In 1994, the World Health Organization/ International Agency for Research on Cancer concluded that *Hp* is a 'definite carcinogen' based on the epidemiological findings (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1994). However the pathogenic roles of *Hp* are still not fully understood. Eradication of *Hp* reduces the relapse rate of peptic ulcers and also results in histological resolution of chronic active gastritis (Hunt, 1996). The standard regimen for this purpose is adoption of triple therapy with a proton pump inhibitor in combination with two antibiotics, clarithromycin and amoxicillin (Misiewicz et al., 1997). Although the currently most effective treatment regimens cure about 90% of infections, 10% of patients remain *Hp* positive. Several factors contribute to treatment failure. These include patient compliance, bacterial resistance to antibiotics, and treatment related issues (Graham, 1998; Huang and Hunt, 1999). Therefore, it is important to find alternative approaches to

[1]Division of Oncological Pathology, Aichi Cancer Center Research Institute, [2]Division of Gastroenterology and Hepatology, Department of Medicine, Toho University School of Medicine, [3]Department of Pathology, Wakayama Medical University, [4]Central Clinical Laboratories, Shinshu University Hospital. Address for correspondence: Tetsuya Tsukamoto, Division of Oncological Pathology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa, Nagoya, 464-8681 Japan. Fax: +81-52-764-2972 E-mail: ttsukamt@aichi-cc.jp

control which are both effective and safe in terms of gastrointestinal protection from *Hp* associated diseases.

Epidemiologic studies overwhelmingly agree on the protective effect of fruits and vegetables in reducing the risk of gastric cancer (Serafini et al., 2002). In Japan, processed foods made from fruits of Prunus mume Sieb. et Zucc. (Japanese apricot or "Ume" in Japanese) are popular and traditionally considered to have miscellaneous medical benefit, such as antibacterial and fungicidal properties (Fujita et al., 2002; Maitani et al., 1985). Nomura et al. (Nomura et al., 1982) previously reported a significant negative association of ume (pickled plum) intake with intestinal metaplasia of the human stomach.

Mongolian gerbils can be easily infected with *Hp*, providing a good experimental animal to clarify the role of *Hp* in chronic active gastritis, peptic ulcers, intestinal metaplasia, and gastric cancer (Hirayama et al., 1996). We have established a gastric carcinogenesis model using these animals, and demonstrated that gastric cancer development is enhanced by *Hp* infection when they are treated with chemical carcinogens, like *N*-methyl-*N*-nitrosourea (MNU) or *N*-methyl-*N'*-nitrosoguanidine (MNNG) (Shimizu et al., 1999; Tatematsu et al., 1998). *Hp* eradication reduces the enhancing effect of *Hp* on gastric carcinogenesis (Cao et al., 2002; Nozaki et al., 2003).

In the present study, we therefore, investigated the efficacy of fruit-juice concentrate of Japanese apricot (CJA) in the glandular stomach of *Hp*-infected Mongolian gerbils.

Materials and Methods

Animals and Samples

A total of 60 specific pathogen-free male, four-week-old Mongolian gerbils (Meriones unguiculatus; MGS/Sea, Seac Yoshitomi, Ltd., Fukuoka, Japan) were housed in steel cages on hardwood-chip bedding in an air-conditioned biohazard room with a 12-h light/12-h dark cycle. They were given food (Oriental CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) irradiated with 30 Gy γ-rays and autoclaved distilled water. The experimental design was approved by the Animal Care Committee of the Aichi Cancer Center Research Institute, and the animals were cared for in accordance with institutional guidelines. CJA was obtained from Minabegawa Village Office (Wakayama, Japan). CJA dissolved in distilled water at concentrations of 1 and 3% was freshly prepared three times per week for administration as drinking water.

Bacteria

Hp strain ATCC 43504 (American Type Culture Collection, Rockville, MD) was inoculated on Brucella agar plates (Becton Dickinson Co., Cockeysville, MD) containing 7% v/v heat-inactivated fetal bovine serum and incubated at 37°C under microaerobic conditions using an Anaero Pack Campylo (Mitsubishi Gas Chemical Co., Inc., Tokyo) at high humidity. Two days later, the bacteria grown on the plates were introduced into Brucella broth (Becton Dickinson Co.) supplemented with 7% v/v heat-inactivated fetal bovine serum and incubated under the same conditions for 24 h. The broth cultures of *Hp* were checked under a phase contrast microscope for bacterial shape and mobility. Samples containing about 1.0×10^8 colony-forming units per milliliter were used as the inoculum and delivered intra-gastrically (i.g.) using an oral catheter to gerbils fasted for 24 h. Uninfected gerbils underwent sham inoculation using the same sterile Brucella broth.

Experimental Protocol

The experimental design is illustrated in Fig. 1. Sixty gerbils were divided into 5 groups. *Hp* was inoculated into three of these groups at 1 experimental week. The other 2 groups received Brucella broth. CJA was administrated to *Hp*-inoculated and *Hp*-free animals in drinking water at the concentrations of 0, 1 or 3%, in all cases until the end of experiment at week 10. The gerbils were killed humanely at the end of the study period. All animals were subjected to deep ether anesthesia after 24 h fasting, laparotomized, and exsanguinated from the inferior vena cava, followed by excision of their stomachs. One half of each glandular stomach was fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) and routinely processed for histopathological examination, and the other half was quick frozen at -70°C for genomic DNA analysis.

Histopathological Analyses

Tissue sections were stained with hemotoxylin and eosin (H&E), Giemsa, and by immunohistochemistry for examination of *Hp* (anti-*Hp* serum, Dako Cytomation, Copenhagen, Denmark). The degree of chronic active gastritis was graded according to criteria modified from the Updated Sydney System (Dixon et al., 1996) by scoring the following parameters: mononuclear cell infiltration (0-3; 0, normal; 1, mild infiltration into lamina propria; 2, moderate infiltration into lamina propria; 3, marked infiltration into lamina propria and multiple lymphoid follicle formation); neutrophil infiltration (0-3; 0, none; 1, number of neutrophils in the pyloric mucosa in a line from the forestomach to the

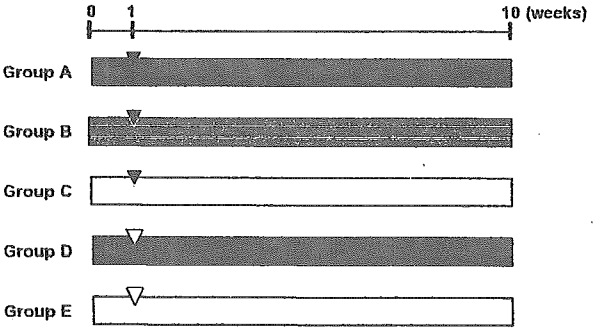


Figure 1. Experimental Design. Four week-old male Mongolian gerbils were used. Intragastric inoculation of Hp (closed triangles) or Brucella broth (open triangle). 3% (closed bar) or 1% (hatched bar) fruit-juice concentrate of Japanese apricot (CJA) was given in the drinking water. Control groups received unsupplemented water (open bar).

Table 1. PCR Primer Sequnces used in the Light Cycler Analysis

Description	Gene	Sequences	Product length (bp)	Accession No.
ua1	Urease A	5'-TGTTGGCGACAGACCGGTTCAAATC-3' (sense)	120	M60398
ua2		5'-GCTGTCCCGCTCGCAATGTCTAAGC-3' (antisense)		
ga1	GAPDH[a]	5'-AACGGCACAGTCAAGGCTGAGAACG-3' (sense)	118	AB040445
ga2		5'-CAACATACTCGGCACCGGCATCG-3' (antisense)		

[a] glyceraldehyde-3-phophate dehydrogenase

duodenum<50/mm; 2, 50-100/mm; 3, >100/mm); *Hp* density (0-3; 0, none; 1, mild *Hp* density; 2, moderate; 3, marked). The thickness of the pyloric mucosa was also measured at five randomly selected points in the foveolar epithelium.

Serology

Serum samples were used to measure the titer of anti-*Hp* IgG antibodies (GAP-IgG; Biomerica, Newport Beach, CA) by enzyme-linked immunosorbent assay (ELISA) using anti-gerbil IgG antibodies. The antibody titer was expressed by means of an arbitrary index (AI). A value greater than 1.37 AI was considered to be positive for *Hp* infection in both the infection and the control groups, as described earlier (Kumagai et al., 2001). Serum gastrin levels were measured using a gastrin radioimmunoassay kit (Gastrin-RIAkit II; Dainabot Co., Ltd., Tokyo).

Real-time Polymerase Chain Reaction and Relative Quantitative Analysis

Genomic DNA was extracted from glandular stomach tisue of gerbils using a DNeasy tissue kit (QIAGEN, Hilden, Germany). For *Hp* quantification, *Hp* specific urease A gene dosage within glandular stomachs of *Hp*-inoculated gerbils, relative quantitative real-time polymerase chain reaction (PCR) of Urease A was performed with the LightCycler system (Roche Diagnostics, Mannheim, Germany), using gerbil specific glyceraldehyde-3-phophate dehydrogenase (GAPDH) gene as an internal control. This was performed basically as described (Tsukamoto et al., 2001; Tsukamoto et al., 2004) using QuantiTect SYBR Green PCR (QIAGEN) with the optimal Mg^{2+} concentration at 2.5mM. The 5'-and 3'-primer sequences are listed in Table 1. Specificity of the PCR reaction was confirmed using the melting program provided with the LightCycler software. To further confirm that there was no obvious primer dimer formation or amplification of any extra bands, the samples were electrophoresed in 3% agarose gels and visualized with ethidium bromide after the LightCycler reaction. Relative quantitative analysis of *Hp* urease A gene expression was performed as earlier established using an internal control without the necessity of external standards (Tsukamoto et al., 2001; Tsukamoto et al., 2004), with values expressed as the percentage urease A gene expression, relative to the 100% in the *Hp*-inoculated control group (group C).

Statistics Analysis

The Mann-Whitney *U* test was applied to establish the significance of differences in urease A gene expression for corrected crossing points, microscopic score for gastritis, mucosal hyperplasia, titers of anti-*Hp* IgG antibodies, serum gastrin levels. *P* values <0.05 were considered to be statistically significant.

Results

Intake of CJA

Data for total intake of CJA per animal are shown in Table 2. CJA administration did not affect food intake or body weights.

Inflammation Score

Table 2 summarizes data for the efficacy of CJA in the glandular stomach of *Hp*-infected Mongolian gerbils. All animals of the *Hp*-inoculated control group (group C)

Table 2 Effects of Fruit-juice Concentrated of Japanese apricot (CJA) on Gastric Lesion of Mongolian Gerbils

Group	Administration	No. of gerbils	Microscopic score [SD]	Mucosal hyperplasia (mm) [SD]	Anti-*Hp* titer titer (AI) [SD]	Serum gastrin (pg/ml) [SD]	Total CJA intake (g/gerbil) [SD]
A	3 % CJA + Hp	20	3.00[a,b] [1.95]	0.34[c] [0.11]	4.01 [2.86]	101.13[d,c] [22.90]	10.54 [0.67]
B	1% CJA + Hp	21	4.38[a] [1.91]	0.42 [0.23]	5.89 [3.36]	133.19 [29.46]	4.76 [0.60]
C	Hp	10	8.00 [1.25]	0.50 [0.23]	6.47 [4.14]	150.31 [40.00]	0
D	3 % CJA	4	0	0.21 [0.02]	0.48 [0.17]	117.88 [18.54]	10.68 0
E	Control	5	0	0.23 [0.03]	0.18 [0.08]	140.88 [26.28]	0

[a] P<0.0001 vs. group C [b] P<0.05 vs. group B [c] P<0.05 vs. group C [d] P<0.005 vs. group C [e] P<0.001 vs. group B

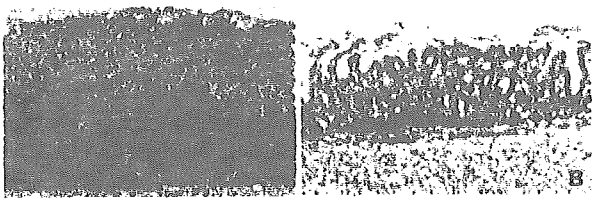


Figure 2. Histopathological Findings in the Pyloric Mucosa of Mongolian Gerbils Inoculated with *Hp*. (A) *Hp*-inoculated control group (group C). The glandular stomach shows hyperplastic change and severe infiltration of inflammatory cells (H&E, Original magnification, x50). (B) *Hp*-inoculated 3% CJA group (group A). The glandular stomach shows mild infiltration of inflammatory cells and mucosal hyperplasia (H&E, Original magnification, x50).

microscopically demonstrated severe gastritis with moderate to marked infiltration of inflammatory cells, mucosal hyperplasia with hemorrhagic erosion and moderate to marked *Hp* density mainly in the pyloric mucosa of glandular stomachs (Fig. 2A). The microscopic scores for the 1 and 3% CJA administrated group (groups A and B) were significantly lower than for the *Hp*-inoculated control group, with dose-dependence (Table 2). The thickness of the pyloric mucosa was also reduced dose-dependently in CJA administrated group, reaching significance in the 3% CJA group (Fig. 2B). No evidence of gastritis and mucosal hyperplasia was found in any *Hp*-free animals.

Antibody Titer and Serum Gastrin Level

Titer of anti-*Hp* antibodies in all *Hp*-inoculated groups were greater than the cut off values expect in one animal in group A, which was excluded from the analysis. There were no significant differences in antibody titers among groups A-C (Table 2). The values for serum gastrin were reduced dose-dependently in the CJA groups, and significantly with the 3% dose (group A) (Table 2).

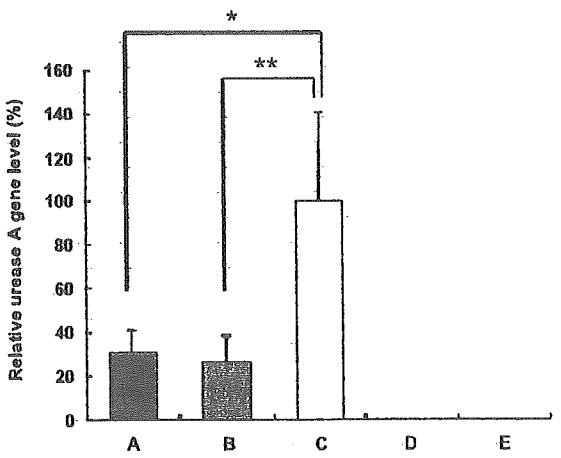


Figure 3. Relative Expression Levels of the Urease A Gene in Glandular Stomachs of Mongolian Gerbils. Values were set at 100% in group C and expressed as mean ± SE. Note decrease in relative urease A gene levels in groups A and B as compared to group C. *P<0.05 and **P<0.01, by the Mann-Whitney *U* test.

Quantification of Hp

Real-time PCR was performed to demonstrate expression of the urease A gene of *Hp*-inoculated groups using GAPDH as an internal control. Average relative urease A gene levels of glandular stomach in 1 and 3% CJA and *Hp*-inoculated control groups were 26.6±11.6% (average ±SE), 30.3±10.5% and 100±40.9%, respectively. The lowering by CJA was significant (P<0.01 and P<0.05, respectively, of 1 and 3%) (Fig. 3). Furthermore, no amplification of the urease A gene was detected in 4 of 20 animals (20%) in group A and 1 of 21 animals (4.8%) in group B, in addition to all the *Hp*-free animals.

Discussion

Our present data provide clear evidence that a fruit-juice concentrate of Japanese plums administered in the drinking water can suppress chronic active gastritis in the glandular stomachs of *Hp*-infected Mongolian gerbils in a dose-dependent manner, reducing urease A gene amount in the *Hp*-inoculated glandular stomach. In the 20% of 3% CJA and 4.8% of 1% CJA administered gerbils without detectable urease A gene, histological examination for *Hp* also proved negative, indicating the possibility that *Hp* had been eradicated in these animals. Rokbi et al. have previously demonstrated that real-time PCR is a powerful tool for the detection and quantification of *Hp* gene expression in the gastric mucosa (Rokbi et al., 2001) and PCR amplification of the *Hp* urease A gene is a highly sensitive and specific method for the diagnosis of *Hp* infection (Clayton et al., 1992).

The Japanese plum (ume), Prunus mume Sieb. et Zucc. (Rosaceae), has been traditionally used as a medical food in Japan and in Chinese traditional medicine, various parts of the plant are used. Although a number of reports have been published with concrete evidence that Japanese apricots are effective against diseases (Maitani et al., 1985), information on the mechanisms, for example of its antibacterial and fungicidal properties, is limited. It has been postulated that antioxidants may reduced cancer risk by modulating red-ox status, by preventing biologic oxidant, and by inhibiting the formation of carcinogen (Serafini et al., 2002). Utsunomiya et al. previously reported that fruit-juice concentrate of Japanese plum possesses a potent antioxidant activity (Utsunomiya et al., 2002). Iimuro et al. have shown that antioxidative effects of garlic may have suppressive effects on *Hp*-induced gastritis in Mongolian gerbils (Iimuro et al., 2002). We therefore hypothesize that antioxidative effects of CJA may have contributed to the suppression of chronic active gastritis in glandular stomach of *Hp*-infected Mongolian gerbils.

In addition, CJA harbors strong acids, including citric and malic acid (Chuda et al., 1999; Fujita et al., 2002), which may exert antibacterial action and cause environmental change in the stomach. Suppressive effects on gastric cancer development would be expected as a result of the decrease of quantity of *Hp* and improvement of *Hp*-induced chronic

active gastritis by administration of CJA. Actual ingredients which might be effective for *Hp*-induced chronic active gastritis have not been clarified but warrant further examination. Studies are now in progress to clarify the suppressive effect of gastric cancer development in gastric carcinogenesis model using Mongolian gerbils.

In conclusion, in this present study, we found CJA to suppress chronic active gastritis in the glandular stomachs of *Hp*-infected Mongolian gerbils. Therefore, CJA may have potential as a safe and inexpensive agent to control *Hp*-associated gastric disorders in Japan, including gastric neoplasia.

Acknowledgments

The authors thank Ms. Hisayo Ban for expert technical assistance. This study was supported in part by a Grant-in-Aid for the Third-term Comprehensive 10-year Strategy for Cancer Control, a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Cao X, Tsukamoto T, Nozaki K, et al (2002). Earlier Helicobacter pylori infection increases the risk for the N-methyl-N-nitrosourea-induced stomach carcinogenesis in Mongolian gerbils. *Jpn J Cancer Res*, **93**, 1293-8.

Chuda Y, Ono H, Ohnishi-Kameyama M, et al (1999). Mumefural, citric acid derivative improving blood fluidity from fruit-juice concentrate of Japanese apricot (Prunus mume Sieb. et Zucc). *J Agric Food Chem*, **47**, 828-31.

Clayton CL, Kleanthous H, Coates PJ, Morgan DD, Tabaqchali S (1992). Sensitive detection of Helicobacter pylori by using polymerase chain reaction. *J Clin Microbiol*, **30**, 192-200.

Dixon MF, Genta RM, Yardley JH, Correa P (1996). Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol*, **20**, 1161-81.

Fujita K, Hasegawa M, Fujita M, et al (2002). Anti-Helicobacter pylori effects of Bainiku-ekisu (concentrate of Japanese apricot juice) (in Japanese). *Nippon Shokakibyo Gakkai Zasshi*, **99**, 379-85.

Graham DY (1998). Antibiotic resistance in Helicobacter pylori: implications for therapy. *Gastroenterology*, **115**, 1272-7.

Hirayama F, Takagi S, Yokoyama Y, Iwao E, Ikeda Y (1996). Establishment of gastric Helicobacter pylori infection in Mongolian gerbils. *J Gastroenterol*, **31 Suppl 9**, 24-8.

Huang JQ, Hunt RH (1999). Treatment after failure: the problem of "non-responders". *Gut*, **45 Suppl 1**, I40-4.

Hunt RH (1996). Eradication of Helicobacter pylori infection. *Am J Med*, **100**, 42S-50S; discussion 50S-51S.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (1994). Schistosomes, liver flukes and Helicobacter pylori. World Health Organization / International Agency for Research on Cancer, Lyon. pp. 177-241.

Iimuro M, Shibata H, Kawamori T, et al (2002). Suppressive effects of garlic extract on Helicobacter pylori-induced gastritis in Mongolian gerbils. *Cancer Lett*, **187**, 61-8.

Kumagai T, Yan J, Graham DY, et al (2001). Serum immunoglobulin G immune response to Helicobacter pylori antigens in Mongolian gerbils. *J Clin Microbiol*, **39**, 1283-8.

Maitani T, Uchiyama S, Saito Y (1985). Determination of cyanogenic compounds in "health foods" made of ume (Japanese apricot) (in Japanese). *Eisei Shikenjo Hokoku*, **103**, 123-5.

Marshall BJ, Warren JR (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, **1**, 1311-5.

Misiewicz JJ, Harris AW, Bardhan KD, et al (1997). One week triple therapy for Helicobacter pylori: a multicentre comparative study. Lansoprazole Helicobacter Study Group. *Gut*, **41**, 735-9.

Nomura A, Yamakawa H, Ishidate T, et al (1982). Intestinal metaplasia in Japan: association with diet. *J Natl Cancer Inst*, **68**, 401-5.

Nomura A, Stemmermann GN, Chyou PH, et al (1991). Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med*, **325**, 1132-6.

Nozaki K, Shimizu N, Ikehara Y, et al (2003). Effect of early eradication on Helicobacter pylori-related gastric carcinogenesis in Mongolian gerbils. *Cancer Sci*, **94**, 235-9.

Rokbi B, Seguin D, Guy B, et al (2001). Assessment of Helicobacter pylori gene expression within mouse and human gastric mucosae by real-time reverse transcriptase PCR. *Infect Immun*, **69**, 4759-66.

Serafini M, Bellocco R, Wolk A, Ekstrom AM (2002). Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology*, **123**, 985-91.

Shimizu N, Inada KI, Tsukamoto T, et al (1999). New animal model of glandular stomach carcinogenesis in Mongolian gerbils infected with Helicobacter pylori and treated with a chemical carcinogen. *J Gastroenterol*, **34 Suppl 11**, 61-6.

Tatematsu M, Yamamoto M, Shimizu N, et al (1998). Induction of glandular stomach cancers in Helicobacter pylori-sensitive Mongolian gerbils treated with N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine in drinking water. *Jpn J Cancer Res*, **89**, 97-104.

Tsukamoto T, Fukami H, Yamanaka S, et al (2001). Hexosaminidase-altered aberrant crypts, carrying decreased hexosaminidase alpha and beta subunit mRNAs, in colon of 1,2-dimethylhydrazine-treated rats. *Jpn J Cancer Res*, **92**, 109-18.

Tsukamoto T, Inada K, Tanaka H, et al (2004). Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. *J Cancer Res Clin Oncol*, **120**, 135-45.

Uemura N, Okamoto S, Yamamoto S, et al (2001). Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med*, **345**, 784-9.

Utsunomiya H, Takekoshi S, Gato N, et al (2002). Fruit-juice concentrate of Asian plum inhibits growth signals of vascular smooth muscle cells induced by angiotensin II. *Life Sci*, **72**, 659-67.

Gastric cancer screening of a high-risk population in Japan using serum pepsinogen and barium digital radiography

Hiroshi Ohata,[1,3] Masashi Oka,[1] Kimihiko Yanaoka,[1] Yasuhito Shimizu,[1] Chizu Mukoubayashi,[1] Kouichi Mugitani,[3] Masataka Iwane,[3] Hideya Nakamura,[3] Hideyuki Tamai,[1] Kenji Arii,[1] Hiroya Nakata,[1] Noriko Yoshimura,[2] Tetsuya Takeshita,[2] Kazumasa Miki,[4] Osamu Mohara[3] and Masao Ichinose[1,5]

[1]Second Department of Internal Medicine and [2]Department of Public Health, Wakayama Medical University, 811-1 Kimiidera, Wakayama-city, Wakayama 641-0012; [3]Wakayama Wellness Foundation, 1850 Minato, Wakayama-city, Wakayama 640-8555; and [4]Division of Gastroenterology and Hepatology, Toho University School of Medicine 5-21-16 Omorinishi, Tokyo 143-8540, Japan

(Received April 1, 2005/Revised July 4, 2005/Accepted July 21, 2005/Online publication August 29, 2005)

With the aim of developing more efficient gastric cancer screening programs for use in Japan, we studied a new screening program that combines serum pepsinogen (PG) testing and barium digital radiography (DR). A total of 17 647 middle-aged male subjects underwent workplace screening over a 7-year period using a combination of PG testing and DR. This program's effectiveness, as well as other characteristics of the program, was analyzed. Forty-nine cases of gastric cancer were detected (comprising 88% early cancer cases). The detection rate was 0.28%, and the positive predictive value was 0.85%. The PG test detected 63.3% of cases, DR detected 69.4% of cases, and both tests were positive in 32.7% of cancer cases. The two methods were almost equally effective, and were considerably more effective than conventional screening using photofluorography. Each screening method detected a distinct gastric cancer subgroup; the PG test efficiently detected asymptomatic small early cancer with intestinal type histology, while DR was efficient at detecting cancers with depressed or ulcerated morphology and diffuse type histology. The cost for the detection of a single cancer was much less than that for conventional screening. In fact, it is possible to further reduce the cost of detecting a single cancer to a cost comparable to that of surgically resecting a single gastric cancer. Thus, it is probable that a highly efficient gastric cancer screening system can be implemented by combining the two screening methods. Such a screening program would be beneficial in a population at high risk for gastric cancer. (*Cancer Sci* 2005; 96: 713–720)

Gastric cancer has been one of the leading causes of cancer-related deaths worldwide and in Japan.[1–4] The Japanese population is at especially high risk for gastric cancer, with a prevalence that is markedly higher than that in other industrialized nations. In Japan, 49 213 people died from gastric cancer in 2002.[5] To cope with this serious public health problem, a gastric cancer screening system using double contrast barium X-ray was introduced in the 1960s throughout Japan. Annually, more than five million people undergo screening and, as a result, thousands of gastric cancers are detected every year.[6–9] However, the present gastric cancer screening system leaves much to be desired; the number of subjects screened has recently been reported to be decreasing, and the screening program itself covers less than 10% of the at-risk population.[10] Furthermore, conventional barium X-ray by photofluorography with a 10 cm × 10 cm sized film is used to screen nearly 80% of the subjects screened annually, so its resolution is by no means high; in fact, it is able to detect no more than 39% of early gastric cancers.[11] Thus, low cost-effectiveness and the risk of X-ray exposure have become issues of concern. Therefore, a more efficient screening system has been sought.

In high incidence areas, such as Japan, stomach carcinogenesis is considered to begin with gastritis that proceeds to extensive atrophy together with intestinal metaplasia, then to dysplasia, and finally to cancer.[12,13] We have previously reported that serum pepsinogen (PG) levels provide a precise measure of the extent of gastric atrophy, and that the serum pepsinogen test is useful in identifying subjects with widespread atrophic gastritis, which is presumed to be a precancerous lesion, especially for intestinal type gastric cancer.[14] The serum PG test is considered to be suitable for cancer screening because it is low-cost, easy to perform, provides quick results, and produces no patient discomfort.[15,16] Recently, serological cancer screening using the PG test followed by endoscopy was introduced on an experimental basis by some local communities and workplaces in Japan.[17–20] This screening strategy has received widespread attention because of its significantly increased cancer detection rate. In addition, the number of subjects undergoing screening has also increased dramatically in the target populations. However, although the PG test is a reliable test for extensive atrophy, it does not directly detect cancer, but only identifies individuals who need further screening by endoscopy. As suggested by various pathological and epidemiological data, the association between atrophic gastritis and intestinal type gastric cancer is very strong, whereas there is only a weak association, if any, between atrophic gastritis and diffuse-type gastric cancer.[21,22] Thus, it is probable that non-gastritis based cancers, especially

[5]To whom correspondence should be addressed.
E-mail: ichinose@wakayama-med.ac.jp

© Japanese Cancer Association doi: 10.1111/j.1349-7006.2005.00098.x

of the diffuse type, escape detection by the PG test. Indeed, as several preliminary reports published by us and others have indicated, quite a few cancers, including those in the advanced stage, tend to be missed by serological cancer screening.[23–25] Thus, it is essential that the design of a stable and efficient cancer screening system includes a strategy that deals with such PG test-negative cancers. Recent reports have indicated that barium X-ray with digital radiography (DR) has a high diagnostic value in the detection of gastric cancers. Therefore, it is considered to be a good alternative to conventional barium X-ray.[26–28] Thus, to overcome the drawbacks of gastric cancer screening using the PG test, we introduced gastric cancer screening using a combination of the PG test and DR as the first-step screening tool. In the present study, we analyzed the results of 7 years of screening, and we evaluated not only the validity of the screening system itself, but also the particular characteristics of each of the two screening methods.

Materials and Methods

The subjects were male employees who underwent gastric cancer screening in a workplace in Wakayama City, which is in the western part of Japan. In 2000, the gastric cancer mortality rate for the area was 45.7/100 000 for men and 18.0/100 000 for women, compared to 39.1/100 000 for men and 15.3/100 000 for women for the whole country. In fact, Wakayama ranks third among the 47 administrative divisions of Japan in terms of gastric cancer mortality.

From April 1995 through to the end of March 2002, a total of 17 647 male subjects (mean age [± SD]: 50.4 ± 5.4 years old; range: 40–60 years old) were screened with a PG test and barium DR. The number of those screened for the first time in 1995 was 3068, and among them, 2160 were screened repeatedly with the two screening tests during the following years. If either or both of the tests were positive, the subjects were further examined by upper gastrointestinal endoscopy (XQ-200, Olympus, Tokyo, Japan). Subjects who required prompt medical care or who had previously undergone gastric resection were excluded and were analyzed separately. Serum PG levels were measured using a modification of the RIA method (Riabeads Kit, Dainabott Co., Tokyo), which we reported on previously.[29] Subjects were screened on the basis of the following PG-test positive criteria: PG I level of less than 50 μg/L and PG I/II ratio of less than 3.0.[30,31] While taking into account the amount of manpower required for conducting thorough endoscopic examinations, the criteria were established so that the sensitivity would be maximized with a positive rate of approximately 20%.

The criteria had a sensitivity of 69% and a specificity of 80%.[30] In the analysis of the PG test results, subjects who had been prescribed any medications that might affect gastrointestinal function (e.g. proton pump inhibitors or non-steroidal anti-inflammatory drugs) prior to the examination were excluded. For upper-gastrointestinal barium X-ray, remote controlled X-ray fluoroscopy (TU-230XB, Hitachi Medico, Japan) and real-time digital radiography (DR-2000H) were used. A total of 150 mL of high concentration barium (200%) was used for the double contrast upper-gastrointestinal X-ray series, and 11 films were taken for each subject. All subjects were followed for the duration of the study period to determine gastric cancer-related deaths. Gastric cancer-related deaths were determined on the basis of information collected about the terminal event. Standardized mortality ratios (SMR) of gastric cancer among participants during the 7-year observation period were calculated based on sex, age, and year-specific gastric cancer mortality, using the male population aged 40–60 years in Wakayama city as the standard population. For the standard population, the expected number of deaths for each 10-year age category was calculated for each of the screening years and then combined to obtain the overall expected number of deaths. Then, the ratio of the observed number of deaths to the expected number of deaths (SMR) during the study period was calculated. The 95% confidence intervals of the SMR were calculated using Poisson regression models.

Data were analyzed using SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA). Differences were tested for statistical significance using Student's *t*-test and analysis of variance for comparisons among multiple values.

The ethics committee of Wakayama Medical University approved the protocol, and informed consent was obtained from all participating subjects.

Results

The results of gastric cancer screening from 1995 through to 2002 were analyzed and are summarized in Table 1. The effectiveness of the two primary screening methods was compared. During the 7-year study period, 17 647 middle-aged male subjects underwent screening. The PG test results were positive in 3441 subjects (19.5%), and DR was positive in 3971 subjects (22.5%). Endoscopy was performed in 2771 (78.8%) of the PG test-positive cases and 3341 (84.1%) of the DR-positive cases. During the study period, 49 cases (52 lesions) of gastric cancer were detected, a detection rate of 0.28%. The positive predictive value of the two combined cancer screening tests was 0.85%. Of the patients with cancer, 63.3% (31 cases) were detected by the PG test, 69.4% (34 cases) by DR, and 32.7% (16 cases) were positive for both screening tests. The two screening methods were almost equally effective, with similar detection rates (PG test,

Table 1. Results of gastric cancer screening

Method	Two screening methods (PG plus DR)	PG	DR
A	17 647		
B	7147 (40.5%)	3441 (19.5%)	3971 (22.5%)
C	5802 (81.2%)	2711 (78.8%)	3341 (84.1%)
D	49	31	34
D/A (%)	0.28	0.18	0.19
D/B (%)	0.69	0.9	0.86
D/C (%)	0.85	1.14	1.02

A, total number of subjects screened; B, total number of subjects requiring further tests (% of the total screened subjects, A); C, total number of the subjects who underwent endoscopy (% of the total subjects who required further tests, B); D, number of cancers detected; PG, pepsinogen test; DR, barium X-ray with digital radiography.

© Japanese Cancer Association doi: 10.1111/j.1349-7006.2005.00098.x

Table 2. Clinicopathological profiles of gastric cancers detected by screening

	I	II	III		I	II	III
Age in years (mean ± SD)	55.5 ± 3.4	54.1 ± 4.4†	53.4 ± 3.6††	Size of the cancer (mm) (mean ± SD)	12.2 ± 7.4	18.8 ± 13.9‡	23 ± 15.9‡‡
No. of cancer cases	15*	18	16				
No. of lesions	18	18	16	Depth of cancer invasion (no. of lesions [%])			
Locus (no. of lesions [%])				Early cancer	18 (100%)	15 (83%)	13(81%)
C	5 (28%)	6 (33%)	2 (13%)	Mucosa	16 (89%)	10 (56%)	8(50%)
M	8 (44%)	9 (50%)	8 (50%)	Submucosa	2 (11%)	5 (27%)	5(31%)
A	5 (28%)	3 (17%)	6 (37%)	Advanced cancer	0 (0%)	3 (17%)	3(19%)
Macroscopic type (no. of lesions [%])				Muscularis Propria	0 (0%)	0 (0%)	1(6%)
IIa	4 (22%)	0 (0%)	1 (6%)	Subserosa	0 (0%)	3 (17%)	2(13%)
IIa+IIc	1 (5%)	0 (0%)	1 (6%)	Histological type (no. of lesions [%])			
IIb	0 (0%)	1 (5%)	0 (0%)	Intestinal type	15 (83%)	10 (56%)	13(81%)
IIb+IIc	0 (0%)	1 (5%)	0 (0%)	Diffuse type	3 (17%)	8 (44%)	3(19%)
IIc	10 (56%)	10 (56%)	10 (64%)	Treatment (no. of lesions [%])			
IIc+III	3 (17%)	3 (17%)	1 (6%)	EMR	8 (44%)	4 (22%)	2(12%)
IIc-like advanced cancer	0 (0%)	0 (0%)	1 (6%)	Surgery	10 (56%)	14 (78%)	14(88%)
Borrmann 2	0 (0%)	1 (5%)	1 (6%)				
Borrmann 3	0 (0%)	2 (12%)	1 (6%)				

PG, pepsinogen test; DR, barium X-ray with digital radiography; I, cancer cases detected only by the PG test; II, cancer cases detected only by barium X-ray with DR; III, cancer cases detected by both methods. C, upper third of the stomach; M, middle third; A, lower third. IIa, superficial-elevated type; IIb, superficial-flat type; IIc, superficial-depressed type. EMR, endoscopic mucosal resection. †Not significant (NS) *vs* I and III; ††NS *vs* I and II; ‡NS *vs* I; ‡‡$P < 0.05$ *vs* I. *Two cases had synchronous mucosal cancers, and one case had metachronous mucosal and submucosal cancers.

0.18%; DR, 0.19%) and positive predictive values (PG test, 1.14%; DR, 1.02%).

The clinicopathological profiles of the detected cancers based on the results of the initial screening method are summarized in Table 2. There was no significant difference in the mean age among the three cancer subgroups (the single PG test-positive group, the single DR-positive group, and the two test-positive group). Of the 52 detected cancer lesions, 46 (88%) were in the early stage (confined to the mucosa or submucosa). The cases detected by the PG test alone were all asymptomatic early cancers, while the cases detected by DR were by no means asymptomatic. In fact, 22% of the cases had some abdominal symptoms. There tended to be a smaller proportion of early cancer among the cases detected by DR alone (83%), and among the cases in whom both tests were positive (81%); the difference from those detected by the PG test was not statistically significant. We found that 89% of the cancers (16 lesions) detected by the PG test alone were localized to the mucosal layer, whereas 56% of those detected by DR alone and 50% of those in whom both screening tests were positive were mucosal cancers. As expected given these findings, the cancers detected by the PG test alone were considerably smaller in size. In particular, the mean (SD) diameter of the cancers detected by a positive PG test was 12.2 (7.4) mm, and for those detected by a positive DR test the mean diameter was 18.8 (13.9) mm, and with both screening methods positive the mean diameter was 23 (15.9) mm; the difference in the diameters between the PG test-positive group and the two test-positive group was statistically significant ($P < 0.05$). Overall, most of the cancers were located in the middle third of the stomach, irrespective of the screening method. However, the cancers detected by DR alone tended to be located more frequently in the upper third of the stomach, where the degree of atrophic change is less severe. According to macroscopic classification, most cancer cases were of the depressed type. Of the 46 early cancer lesions, 37 (80.4%) were IIc or IIc + III, and all but one of the advanced cancers were either Borrmann 2 or 3. The remaining case was a IIc-like advanced cancer. Elevated IIa lesions were detected in 22% of the cases that were PG test-positive alone, 0% of the cases that were DR positive alone, and 6% of cases in which both tests were positive. Of the two histological types of gastric cancer, the intestinal type was the most prevalent detected on screening. This tendency was quite pronounced in the cases detected by the PG test alone, as 83% of these cancers were of the intestinal type, while the proportion of the diffuse type cancers was the highest in those identified by DR alone (44%). Therefore, the PG test is good at detecting small, early, intestinal type cancers among asymptomatic subjects, which tend to escape screening by barium X-ray.

With respect to cancer therapy, all the cancer cases were successfully treated either surgically or endoscopically. Since endoscopic mucosal resection (EMR) is less invasive and produces a better quality of life compared with surgical resection, it has been widely accepted in Japan as the primary

Table 3. Cost-effectiveness analysis of gastric cancer screening according to the screening methods used

Method	Two screening methods (PG plus DR)	PG	DR
Total number of subjects screened	17 647		
Total number of subjects undergoing further tests	5 802	2 711	3 341
Number of cancer cases detected	49	31	34
Total cost (¥)	198 955 000	70 537 000	131 668 000
Cost for detecting a single cancer (¥)	4 060 306	2 275 387	3 872 588

PG, pepsinogen test; DR, barium X-ray with digital radiography. Cost for PG test: ¥2000/subject; cost for barium X-ray with DR: ¥5000/subject; cost for endoscopy: ¥13 000/subject.

therapeutic strategy for the treatment of early gastric cancer. EMR is particularly indicated for intestinal type mucosal cancers of the elevated type no larger than 2 cm in size and for cancers of the depressed type no larger than 1 cm without ulceration.[32] Many cancer cases detected by the PG test alone were small mucosal cancers with no sign of lymph node or distant metastasis, and thus were especially well suited for EMR. As a result, 44% of the cases detected by the PG test alone underwent EMR, and this percentage was higher than in the other two groups: EMR was used to treat 22% of gastric cancers in the single DR-positive group and 12% in the two test-positive group.

Table 3 shows the costs for the cancer screening program using the two methods. Taking into account both the primary and secondary screening costs, the total cost for the present screening approach was ¥198 955 000, which included ¥35 294 000 for the initial PG test screening, ¥88 235 000 for the DR, and ¥75 426 000 for endoscopy. Using the present screening approach, the cost to find a single cancer case was ¥4 060 306. Using the PG test alone, the cost to find a single cancer case was ¥2 275 387, while the calculated cost to find a single cancer case by using the DR method alone was ¥3 872 588.

Figure 1 shows examples of gastric cancer cases detected by the PG test alone. Case (a) had a 10-mm type IIa lesion located in the anterior wall of the gastric angle, and case (b) had an 8-mm type IIa lesion located in the lesser curvature of the proximal antrum. These cases had an intestinal type mucosal cancer and were successfully treated by EMR. Case (c) had a 5-mm IIc + III lesion located in the anterior wall of the lower gastric body. Partial gastrectomy was performed, and pathological examination of the resected stomach revealed a minute, diffuse type mucosal cancer. It is noteworthy that these three cases had been essentially symptom-free and that the DR did not document any abnormalities that would indicate the existence of these lesions. In these three cases no recurrence has been observed to date. These cases clearly indicate that the PG test is especially useful in detecting minute lesions that escape diagnosis by barium X-ray.

Figure 2 shows examples of gastric cancer cases detected only by DR. Case (a) had a 10-mm type IIc + III lesion located in the lesser curvature of the gastric angle. A partial gastrectomy was performed, and pathology revealed a diffuse type mucosal cancer. Currently, no recurrence has been observed. Case (b) had a 30-mm type IIc + III lesion located in the greater curvature of the gastric angle. A partial gastrectomy was performed, and the resected stomach revealed a diffuse type submucosal cancer. Case (c) had a 45-mm Borrmann 3 lesion located in the gastric cardia. Total gastrectomy was performed, and pathology revealed a large, ulcerated, advanced intestinal type cancer invading the subserosa. There have been no signs of recurrence in any of these three cases to date. In these three cases, although they were not symptom-free at screening, the lesion could have been missed if the primary screening was conducted using the PG test alone, because the background mucosa was not atrophic, and thus, the serum pepsinogen level was high. These cases clearly show that gastric cancers, irrespective of stage, can easily escape detection by the PG test alone; for such cancers, barium X-ray is the preferred screening strategy.

In the workplace in which the screening was done, six employees died from gastric cancer during the period from 1991 to 1994 when cancer screening was conducted by conventional barium X-ray using photofluorography. With the introduction of the new screening program, there was no significant reduction in the number of gastric cancer deaths during the first 4 years between 1995 and 1998, as seven employees died. The SMR (95% confidence interval) of gastric cancer in the workplace compared with the same aged male population living in the same area during the period was 2.74 (1.20–5.92). However, in the following 3 years (1999–2001), there was a drastic reduction in cancer deaths, with only one employee dying from gastric cancer; the SMR for this period decreased to 0.87 (0.22–2.76).

Discussion

Previous seroepidemiological studies have clearly indicated a significant correlation between gastric cancer mortality and the prevalence of extensive atrophic gastritis as determined by serum PG testing.[33,34] Therefore, PG test-positive subjects are considered to constitute a population at high risk for gastric cancer, and the PG test is a valuable tool for selecting a population that needs further examination by endoscopy. It has also been reported that barium X-ray with DR is superior to conventional barium X-ray in image quality and diagnostic accuracy for detecting gastric cancer, especially when a high concentration barium meal is used.[35,36] Furthermore, DR has the following advantages over the conventional screen-film system: (i) digital imaging allows image quality to be optimized and radiation dose to be reduced; (ii) DR provides rapid data acquisition, digital image processing, and instant image display; and (iii) DR is compatible with a picture archiving and imaging network.[26–28] Given these features, DR can be particularly useful for barium X-rays using the double-contrast imaging method.[26–28] To improve the

© Japanese Cancer Association doi: 10.1111/j.1349-7006.2005.00098.x

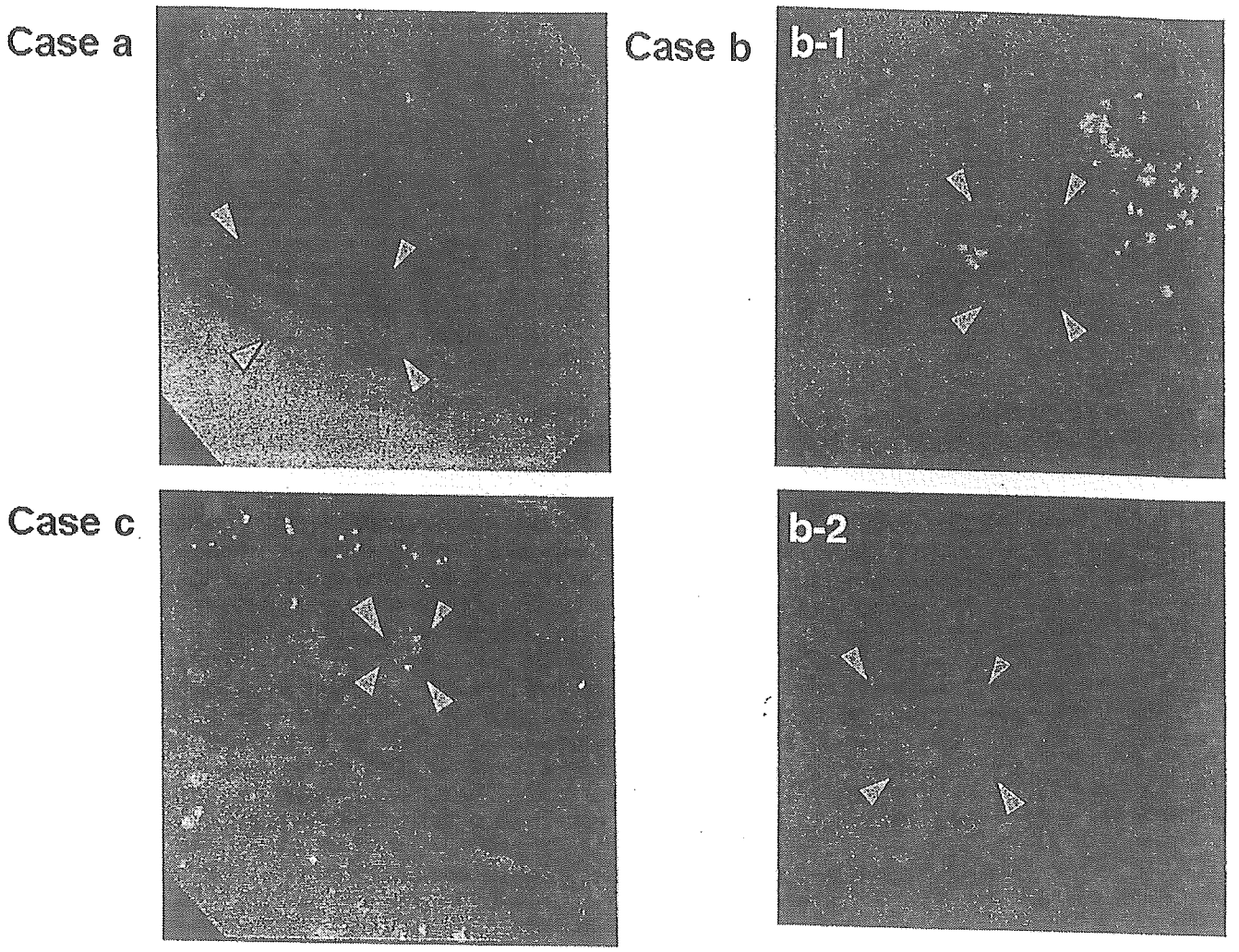


Fig. 1. Representative gastric cancer cases in which only the pepsinogen (PG) test was positive. The PG test efficiently detected small early cancers, especially those with an elevated type morphology and intestinal type histology. (a) A 10-mm type IIa lesion located in the anterior wall of the gastric angle (arrow heads). The serum PG levels were: PGI 27.2 μg/L, PGII 22.6 μg/L, and the PGI/PGII ratio was 1.2. (b) An 8-mm type IIa lesion located in the lesser curvature of the proximal antrum as indicated by the arrow heads (b-1). The indigo carmine dye-spraying method revealed the existence of the tiny elevated lesion more clearly as shown by the arrow heads (b-2). The serum PG levels were: PGI 39.5 μg/L, PGII 39.5 μg/L, and the PGI/PGII ratio was 1. (c) An 8-mm type IIc + III lesion located in the anterior wall of the lower gastric body (arrow heads). The serum PG levels were: PGI 48.5 μg/L, PGII 30.3 μg/L, and the PGI/PGII ratio was 1.6. All three cases had been essentially symptom-free at detection. (a) and (b) were intestinal type mucosal cancers and were successfully treated by endoscopic mucosal resection (EMR). (c) was treated with a partial gastrectomy, and pathological examination revealed a diffuse type mucosal cancer. In all these cases, the background mucosa revealed extensive atrophy, as would be expected with a positive PG test.

efficiency of gastric cancer screening, we introduced the PG test and the barium X-ray with DR as initial screening tools. The results of the present study clearly indicate that both the PG test and DR effectively detect gastric cancer. According to the 2001 annual report of gastric cancer screening prepared by the Japanese Association of Gastrointestinal Mass Screening, the cancer detection rate of nationwide screening using the conventional barium X-ray method was 0.10%.[10] Thus the 0.28% cancer detection rate found in the present screening over a 7-year period was considerably higher than that of the nationwide gastric cancer screening program using conventional barium X-ray examination. Based on their positive predictive values, the PG test and DR are almost equally efficient, and both of them are superior to conventional barium X-ray examination. The detection rate is 1.8 times higher with the PG test and 1.9 times higher with DR than with conventional barium X-ray examination.

In the workplace where this study was done, cancer screening by using the conventional barium X-ray technique with photofluorography had been done until the present screening system was introduced. In the initial year, the cancer detection rates of the PG test and DR were at the same high level (0.38%). The mean detection rates of the two methods for the first 3 years and the next 4 years were: PG test, 0.28% first 3 years, 0.14% next 4 years; DR, 0.29% first 3 years, 0.17% next 4 years. Because the PG test was a new type of screening test applied for the first time in that workplace, the test may have detected prevalent cancers in the

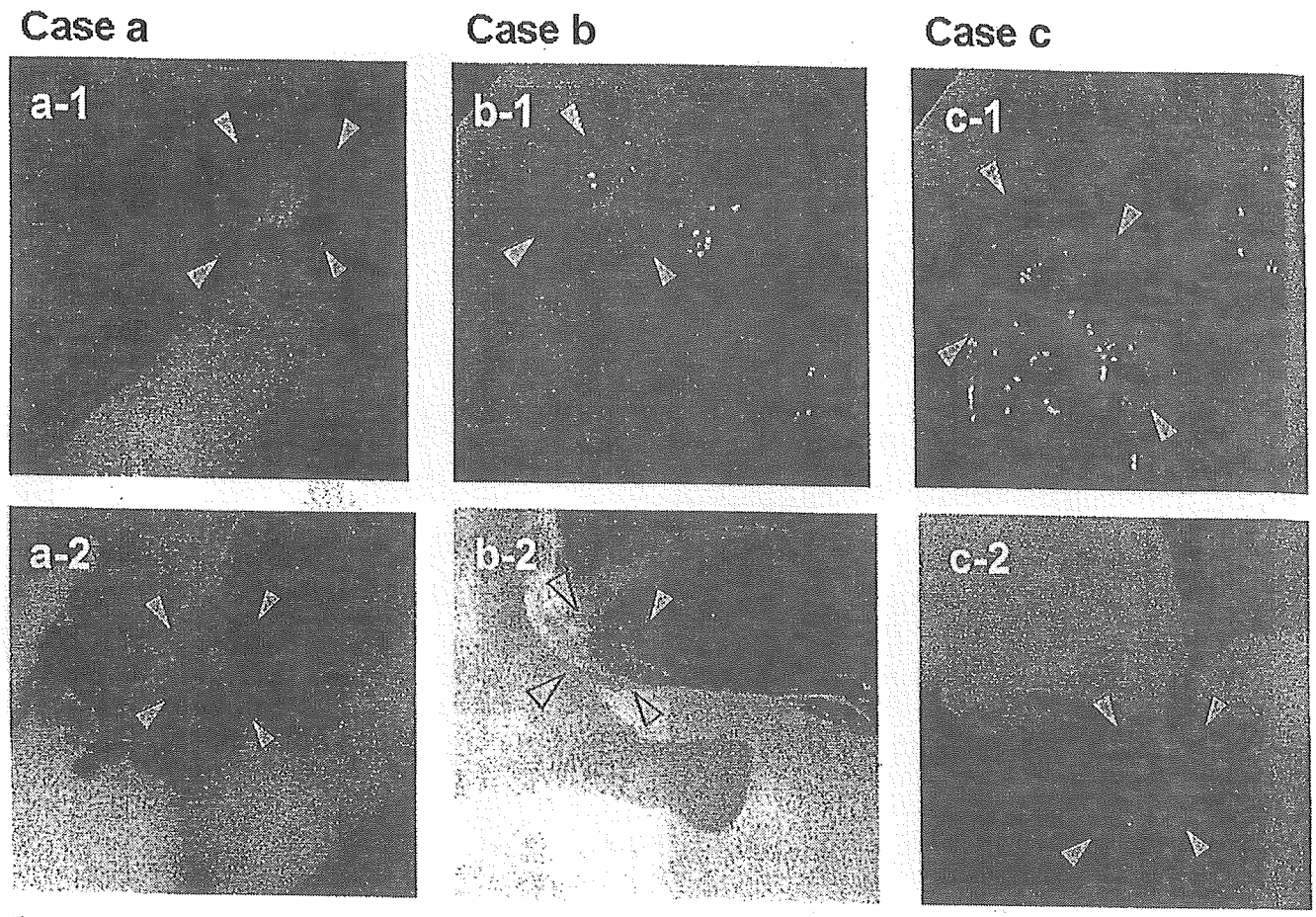


Fig. 2. Representative gastric cancer cases in which only barium digital radiography (DR) was positive. The DR efficiently detected cancers with depressed or ulcerated type morphology and diffuse type histology. Images marked 1 show endoscopic views of each case, and images marked 2 show the DR images of each case. (a) A 10-mm type IIc + III lesion located in the lesser curvature of the gastric angle. Serum PG levels were: PGI 44.6 μg/L, PGII 13.5 μg/L, and the PGI/PGII ratio was 3.3. (b) A 45-mm Borrmann 3 lesion located in the gastric cardia. Serum PG levels were: PGI 43.1 μg/L, PGII 5.9 μg/L, and the PGI/PGII ratio was 7.3. (c) A 30-mm type IIc + III lesion located in the greater curvature of the gastric angle. Serum PG levels were: PGI 77.9 μg/L, PGII 22.9 μg/L, and the PGI/PGII ratio was 2.6. (a) and (c) were both treated by partial gastrectomy, and both had diffuse type histology. (a) was a mucosal cancer, and (c) was a submucosal cancer. (b) underwent a total gastrectomy, and the pathology revealed a large ulcerated advanced intestinal type cancer invading the subserosa. In all these cases, the background mucosa was not atrophic, which would be expected with a negative PG test.

initial year. Thus, it can be expected that by repeating the PG test screening during subsequent years, the detection rate of the serum test would decrease until it finally detected only the annual incident cancers. In contrast, since DR screening was essentially the same type of screening that had already been done annually, it was quite unexpected that the detection rate would be high in the first year of screening, and that it would then decrease in subsequent years. It is therefore likely that, in the first year, DR detected cancers missed by conventional screening, and in the following years, the DR detection rate, although it did decrease, remained higher than that of the conventional barium X-ray screening approach. This clearly indicates the higher sensitivity of DR in comparison with conventional barium X-ray. Thus, the sensitivity of the two screening methods is almost equivalent, both in the initial phase of screening and on subsequent screening, indicating that the combined approach is much better than conventional screening using photofluorography.

In our screening, the overlap between the cancers detected by the PG test and by DR was relatively small, accounting for only 32.7% of the total cancer cases detected. The PG test is a sensitive and specific indicator of extensive mucosal atrophy, while DR is a method that directly visualizes mucosal abnormalities. As could be expected from the difference in the diagnostic mechanisms of the two screening methods, the PG test efficiently detected small early cancers with elevated type morphology and intestinal type histology that tended to be derived from atrophic gastric mucosa. In contrast, DR was efficient at detecting cancers with a depressed or ulcerated type morphology and a diffuse type histology. PG test-positive cancers are usually asymptomatic, which is a reflection of reduced acid secretion due to extensive atrophic gastritis. However, DR-positive cancers, of which approximately a half are PG test-negative and in whom acid secretion is not impaired, are by no means asymptomatic. In our study, cases detected only by the PG test were all asymptomatic early

© Japanese Cancer Association doi: 10.1111/j.1349-7006.2005.00098.x

gastric cancers, 83% of which were intestinal type. Macroscopically, 22% of these cases had elevated morphology, and 89% were limited to the mucosa. These small intestinal type mucosal cancers, especially the elevated type, are particularly well suited for EMR. Thus, the PG test is a screening method that can contribute greatly to the patients' quality of life (QOL) by detecting cancer in its early stages. In other words, the PG test is especially good at screening asymptomatic subjects, whereas symptomatic subjects or PG test-negative subjects should be screened by barium X-ray examination.

In 2002, the Japanese Association of Gastroenterological Mass Survey reported that 5 843 904 subjects underwent mass screening for gastric cancer nationwide, and that 379 965 underwent further examination.[10] Given that initial screening with conventional barium X-ray cost ¥3500 per subject, and endoscopy cost ¥13 000 per subject, the total cost for the screening program was estimated as ¥25 393 209 000 per year. During screening in 2002, 5410 subjects were identified as having gastric cancer. Based on these data, the cost required to find a single case of gastric cancer can be estimated as ¥4 408 543, simply by dividing the total cost by the number of detected cancers. In contrast, using the screening system presented in this paper it would cost ¥4 060 306 to find a single gastric cancer case. Using the PG test alone, the cost to find a single cancer case was ¥2 275 387, whereas it cost ¥3 872 588 to find a single gastric cancer case using DR alone. Both of these screening methods were found to be more cost-effective than conventional barium X-ray. Furthermore, with the introduction of DR, there has been a reduction in costs for films, reagents for development, and waste disposal.[26–28] The total cost over 7 years for cancer screening using barium X-ray was ¥88 235 000 (¥5000/subject), which includes the depreciation cost for the DR system (¥4 000 000/year), the cost for the optical disk (¥250 000/year), hard copy paper (¥220 000/year), the cost for barium meals, effervescent granules, anticholinergics (¥1714 280/year, ¥680/subject) and other costs, including personnel expenses. Since the cost of DR can be decreased considerably, the total cost of DR could be reduced to less than ¥3500/subject, in which case the cost to detect a single case of cancer using a DR screening program would be reduced to less than ¥3 094 044. Furthermore, the PG test cost could be reduced to at least ¥1000/subject, so that the cost would be ¥1 706 129 per cancer using the PG test alone. Thus, it would cost ¥3 159 949 per cancer for a screening program using both the PG test and DR. These figures are comparable to the cost of doing a surgical resection of a stomach cancer in Japan, for which the costs have been calculated as ¥3 417 750 ± 19 982 per patient in patients with preoperative disorders or complications and ¥1 534 070 ± 280 560 per patient in patients without any preoperative disorders or complications.[37] In order to establish a stable and efficient cancer screening system, it is important to optimize the economic aspect of the screening test program.[38] However, neither the PG test nor DR can be recommended as a single screening method for the general population, because, as has been shown, more than 30% of the cancers cannot be detected when these screening methods are used separately. Asymptomatic early cancers, a considerable number of which are particularly suitable for minor invasive endoscopic mucosal resection, escape diagnosis with DR screening, while the PG test cannot detect non-gastritis based cancers, especially those with a depressed type morphology and diffuse type histology, including those in the advanced stage, which can be easily detected by barium X-ray and which have a higher malignant potential than PG test-positive cancers. Because each of the two screening methods detect a distinct subgroup of gastric cancer with distinct clinicopathological characteristics, the combination of the two methods has greatly improved screening sensitivity and is more cost effective than conventional screening.

With the introduction of the new two test screening system, the number of gastric cancer deaths has decreased markedly, and there has been a steady decrease over time in the SMR (using the same aged male population living in the same area as the standard). As yet, the reduction in cancer mortality has not been significant, although it is noteworthy that there have been only two cancer deaths since 1999 in this workplace: one cancer death occurred in a patient who skipped the screening, and the other underwent gastrectomy in 1990 and was thereafter excluded from the screening program and followed separately. Thus, there have been no cancer deaths in the population screened for the last 5 years, and it is thus highly probable that our cancer screening system has successfully reduced gastric cancer mortality.

The next improvement of the present screening system will depend on target setting. Recent studies have clearly indicated that *H. pylori* infection and the resulting severe atrophic gastritis together with intestinal metaplasia are involved in the development of gastric cancer. By following approximately 5000 male subjects of cancer-susceptible age for more than 7 years, we have found that it is quite rare for gastric cancer to develop in *H. pylori*-free stomachs.[39] In Japan today, 20–30% of the middle-aged population (40–60 years of age) is *H. pylori* free, and it is highly probable that subjects from this group, if asymptomatic, could be excluded from screening. In contrast, PG test-positive subjects are at high risk of developing cancer, and according to our data the cancer incidence in this group is 255/100 000 person-years.[39] This incidence increases in a stepwise manner with the progression of atrophic gastritis and reaches 871/100 000 person-years in the group with severe atrophic gastritis and extensive intestinal metaplasia (metaplastic gastritis), which can be easily identified because they are PG test-positive and *H. pylori* antibody-negative.[39] Such cancer high-risk groups, especially the metaplastic gastritis group, should be the target of regular screening by endoscopy. However, in target setting we should pay special attention to the PG test-negative, *H. pylori* antibody-positive group: the cancer incidence in this group is by no means low (107/100 000 person-years), and approximately half of the diffuse cancers that are clinically and biologically more malignant occur in this group. Therefore, this group should also be included in the screening program, and DR should be used to screen this group. Thus, initial screening requires the combination of the PG test and barium DR.

In conclusion, the present results strongly indicate that the establishment of a cancer screening system with a high efficacy is possible by using a combination of the PG test and barium DR. The best way to combine the two methods needs

further investigation, and this may vary significantly depending upon the target population. It is probable that such screening would be beneficial not only in Japan but also in other high risk areas outside Japan, such as East Asia, Central and South America, and Eastern Europe.

Acknowledgment

This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare of Japan.

References

1 Correa P. The epidemiology of gastric cancer. *World J Surg* 1991; **15**: 228–34.
2 Terry MB, Gaudet MM, Gammon MD. The epidemiology of gastric cancer. *Semin Radiat Oncol* 2002; **12**: 111–27.
3 Research Group for Population-Based Cancer Registration in Japan. Cancer incidence rates in Japan in 1997: estimates based on data from 12 population-based cancer registries. *Jpn J Clin Oncol* 2002; **32**: 318–22.
4 Fuchs CS, Mayer RJ. Gastric carcinoma. *N Engl J Med* 1995; **333**: 32–41.
5 Ministry of Health, Labour and Welfare. *Vital Statistics of Japan, Statistics and Information*, vol. 1. Tokyo: Minister's Secretariat, Ministry of Health, Labour and Welfare, 2002.
6 Fukao A, Tsubono Y, Tsuji I, Hisamichi S, Sugahara N, Takano A. The evaluation of screening for gastric cancer in Miyagi Prefecture, Japan: a population-based case-control study. *Int J Cancer* 1995; **60**: 45–8.
7 Murakami R, Tsukuma H, Ubukata T *et al.* Estimation of validity of mass screening program for gastric cancer in Osaka, Japan. *Cancer* 1990; **65**: 1255–60.
8 Hisamichi S. Screening for gastric cancer. *World J Surg* 1989; **13**: 31–7.
9 Kawai K. Screening for gastric cancer in Japan. *Clin Gastroenterol* 1978; **7**: 605–22.
10 Koga M, Miyakawa K, Ikeda S. *Annual Report of Gastroenterological Mass Survey in Japan, 2002.* Tokyo: Japanese Society of Gastroenterology Mass Survey, 2004.
11 Nishizawa M. Present status and prospects for cancer screening. *J Gastroenterol Mass Survey* 1993; **78**: 100–3. (In Japanese.)
12 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735–40.
13 Correa P. A human model of gastric carcinogenesis. *Cancer Res* 1988; **48**: 3554–60.
14 Miki K, Ichinose M, Shimizu A *et al.* Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol Jpn* 1987; **22**: 133–41.
15 Miki K, Ichinose M, Kawamura N *et al.* The significance of low serum pepsinogen levels to detect stomach cancer associated with extensive chronic gastritis in Japanese subjects. *Jpn J Cancer Res* 1989; **80**: 111–4.
16 Ichinose M, Yahagi N, Oka M, Ikeda H, Miki K, Omata M. Screening for gastric cancer in Japan. In: Wu GY, Aziz K, eds. *Cancer Screening*. Totowa, New Jersey: Humana Press, 2001; 255–68.
17 Miki K, Morita M, Sasajima M, Hoshina R, Kanda E, Urita Y. Usefulness of gastric cancer screening using the serum pepsinogen test method. *Am J Gastroenterol* 2003; **98**: 735–9.
18 Hattori Y, Tashiro H, Kawamoto T, Kodama Y. Sensitivity and specificity of mass screening for gastric cancer using the measurement of serum pepsinogens. *Jpn J Cancer Res* 1995; **86**: 1210–5.
19 Yoshihara M, Sumii K, Haruma K *et al.* The usefulness of gastric mass screening using serum pepsinogen levels compared with photofluorography. *Hiroshima J Med Sci* 1997; **46**: 81–6.
20 Kitahara F, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. *Gut* 1999; **44**: 693–7.
21 Ngayo T. Precursors of human gastric cancer. Their frequencies and histological characteristics. In: Farber E, ed. *Pathophysiology of Carcinogenesis in the Digestive Organs*. Baltimore: University Park Press, 1977; 151–60.
22 Correa P. Chronic gastritis and gastric cancer. In: Ming SC, ed. *Precursors of Gastric Cancer*. New York: Praeger Publishers, 1984; 105–16.
23 Miki K, Ichinose M. Serum pepsinogen I level in patients with stomach cancer: its value and limitation in clinical use. *Gan Kagaku Ryoho* 1989; **16**: 1122–8. (In Japanese.)
24 Yahagi N, Shimizu Y, Ichinose M, Miki K, Omata M. Corroborative evidence on mass screening method using serum pepsinogen test for long term follow up of gastric cancer patients. *Clin Gastroenterol* 2002; **17**: 1577–83. (In Japanese with English abstract.)
25 Matsumoto J, Arai T, Yahagi K, Hashimoto Y, Masuda J. A study of serum pepsinogen test method negative gastric cancer. *J Gastroenterol Mass Surv* 2002; **40**: 20–7. (In Japanese.)
26 Iinuma G, Ushio K, Ishikawa T, Nawano S, Sekiguchi R, Satake M. Diagnosis of gastric cancers: comparison of conventional radiography and digital radiography with a 4 million-pixel charge-coupled device. *Radiology* 2000; **214**: 497–502.
27 Sugino Y, Imai Y, Fujisawa H, Hiramatsu K, Amoh H, Kumakura K. Clinical usefulness of digital radiography in the gastrointestinal tract: efficacy of magnification method. *J Digit Imaging* 1995; **8**: 84–8.
28 Takahashi M, Ueno S, Yoshimatsu S *et al.* Gastrointestinal examinations with digital radiography. *Radiographics* 1992; **12**: 969–78.
29 Ichinose M, Miki K, Furihata C *et al.* Radioimmunoassay of serum group I and group II pepsinogens in normal controls and patients with various disorders. *Clin Chim Acta* 1982; **126**: 183–91.
30 Miki K, Ichinose M. Chronic atrophic gastritis and serum pepsinogen levels. *Jpn J Cancer Clin* 1992; **38**: 221–9. (In Japanese with English abstract.)
31 Miki K, Ichinose M, Ishikawa KB *et al.* Clinical application of serum pepsinogen I and II levels for mass screening to detect gastric cancer. *Jpn J Cancer Res* 1993; **84**: 1086–90.
32 Nishi M, Ishihara S, Nakajima T, Ohta K, Ohyama S, Ohta H. Chronological changes of characteristics of early gastric cancer and therapy: experience in the Cancer Institute Hospital of Tokyo, 1950–94. *J Cancer Res Clin Oncol* 1995; **121**: 535–41.
33 Fukao A, Hisamichi S, Osato N. Correlation between the prevalence of gastritis and gastric cancer in Japan. *Cancer Causes Control* 1993; **4**: 17–20.
34 Kabuto M, Imai H, Tsugane S. Correlation between atrophic gastritis prevalence and gastric cancer mortality among middle aged-man in 5 areas in Japan. *J Epidemiol* 1993; **3**: 35–9.
35 Asazaki M, Hirukawa K, Chiyasu S *et al.* Optimal system of the digital radiography on mass survey (computed radiography *vs* digital radiography). *J Gastroenterol Mass Surv* 2000; **38**: 107–17. (In Japanese.)
36 Tsukeshiba I, Iinuma M, Niwa Y, Goto H, Segawa K. Assessment of high-density barium for X-ray examination of the upper gastrointestinal tract using digital radiography. *J Gastroenterol Mass Surv* 2000; **40**: 155–9. (In Japanese.)
37 Kiyama T, Tajiri T, Yoshiyuki T *et al.* An economic evaluation of gastrectomy. *Jpn J Cancer Clin* 2004; **50**: 187–90. (In Japanese.)
38 Tsuji I, Fukao A, Sugawara N, Shoji T, Kuwajima I, Hisamichi S. Cost-effectiveness analysis of screening for gastric cancer in Japan. *Tohoku J Exp Med* 1991; **164**: 279–84.
39 Ohata H, Kitauchi S, Yoshimura N *et al.* Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. *Int J Cancer* 2004; **109**: 138–43.

© Japanese Cancer Association doi: 10.1111/j.1349-7006.2005.00098.x

Early Detection of Asymptomatic Gastric Cancers Using Serum Pepsinogen Levels to Indicate Endoscopic Submucosal Dissection for Better Quality of Life

M. Fujishiro[1], N. Yahagi[1], N. Kakushima[1], S. Kodashima[1], M. Nakamura[1], K. Kobayashi[1], T. Hashimoto[1], N. Yamamichi[1], A. Tateishi[1], H. Miyoshi[1], Y. Moriyama[1], Y. Shimizu[2], M. Oka[2], M. Ichinose[2], M. Omata[1] and K. Miki[3]

[1]Department of Gastroenterology, The University of Tokyo, Tokyo, Japan; [2]Second Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan; [3]Department of Gastroenterology, Toho University, Tokyo, Japan

Summary

Serum pepsinogen (PG) levels correlate closely with a precancerous condition of developing gastric cancer, which can detect asymptomatic gastric cancer patients from blood samples. Endoscopic submucosal dissection (ESD) can be indicated, if the cancer is intramucosal and intestinal-type. In 3,803 healthy individuals (a ratio of man to woman = 2.7, a mean age of 44.5 years) at a workplace, 834 (22%) resulted in positive PG tests (PGI $\leq$ 70 ng/ml, PGI / PGII $\leq$ 3). Gatroscopy was practically performed for 543 (14.3%), which revealed 6 cancers in 5 individuals. Three cancers were curatively resected by ESD and the other 3 were by gastrectomy with lymph node dissection. Combination between serum PG levels and ESD, if possible, may be an attractive strategy for gastric cancer patients to achieve better quality of lives.

Introduction

Serum pepsinogen (PG) levels provide a good surrogate marker for the extent of atrophic gastritis, which is considered to be a precancerous condition of developing gastric cancer. If the PGI level $\leq$ 70 ng/ml and the ratio of PGI to PGII $\leq$3 are defined as the positive PG test, that is

©2005 by MEDIMOND S.r.l.

extensive atrophic gastritis, it is reported that the sensitivity, the specificity, and the positive predictive value of gastric cancer detection in Japanese asymptomatic subjects were around 80%, 70%, and 1.5%, respectively. (1, 2) It is important to detect gastric cancers in their early stages in order to get better survival. Asymptomatic gastric cancer patients may be detected in early stages, measuring serum PG levels from blood samples in asymptomatic individuals at a workplace. Furthermore, a newly developed endoscopic resection technique, that is endoscopic submucosal dissection (ESD), enables us to resect large or ulcerative lesions en bloc. (3-5) If the cancers are resected en bloc and the histological evaluation reveals the cancers' conditions of node-negative tumors, then, curative resection is obtained and a gastrectomy with lymph node dissection is avoided to get a better quality of life (QOL). In this study, we investigated the feasibility of measuring serum PG levels in asymptomatic individuals at a workplace for detection of gastric cancers and the possibility of application of ESD for the detected cancers by positive PG tests.

Materials and Methods

Serum PG levels were measured in 3,803 asymptomatic individuals at a workplace as a primary screening between April 2003 and March 2004. They consisted of 2,783 men and 1,020 women, with a mean age of 44.5 years. Blood samples were taken after fasting, and serum PG levels were assayed by a modified radioimmunoassay method using PGI and PGII Riabeads Kits (Dinabot Co Ltd, Tokyo, Japan). (6) Those with a PGI $\leq$70 ng/ml and PGI / PGII $\leq$3 were defined as having a positive PG test and were recommended to undergo gastroscopy. Gastroscopy was performed at a clinic of the workplace with topical pharyngeal lidocaine anesthesia by three skilled endoscopists, using GIF XQ260 (Olympus, Tokyo, Japan). If endoscopical biopsies taken from suspicious changes of cancers revealed the histological diagnosis of cancers, then the individuals were referred to the affiliated hospitals where ESD could be done, considering the patients' desires. ESD was planed, if the detail check-ups of the cancers revealed that they were considered to be the following conditions; 1. intramucosal, intestinal-type, and no ulcerative findings, 2. intramucosal, intestinal-type, less than 3cm in size, and ulcerative findings. (7) Except for the above conditions, gastrectomy with lymph node dissection was planed, if there was no distant metastasis. Histological diagnosis of the resected specimens by ESD was out of the above and/or with vessel infiltration, and then, an additional gastrectomy with lymph node dissection was performed. The procedures of ESD were carried out under conscious sedation in an endoscopy room, using a single channel upper gastrointestinal endoscope with water-jet system (EG-2931; Pentax, Tokyo, Japan, or

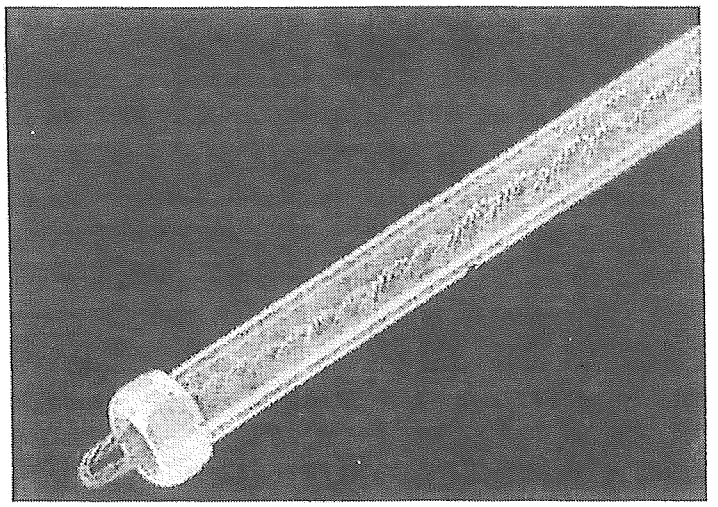


Figure 1. Flex knife.

XGIF-1T240M (prototype); Olympus, Tokyo, Japan) and a high-frequency generator with automatically controlled system (Endocut mode) (Erbotom ICC 200; ERBE, Tübingen, Germany). The procedures consist of five steps; marking, submucosal injection, mucosal incision, submucosal dissection, and treatment of the artificial ulcer. We used a flex knife as a electrosurgical knife (8) (Figure1)and a mixture of a 1% 1900 KDa hyarulonic acid preparation (Suvenyl™, Chugai Pharmaceutical Co., Tokyo, Japan) and 10% glycerin with 5% fructose and 0.9% saline preparation (Glyceol™, Chugai Pharmaceutical Co., Tokyo, Japan) with a small amount of epinephrine (1:200000) as a submucosal injection solution. (9)

Results

Among 3,803 individuals, 834 (22%) (558 men and 276 women) resulted in positive PG tests and 543 (14.3%) (364 men and 179 women)

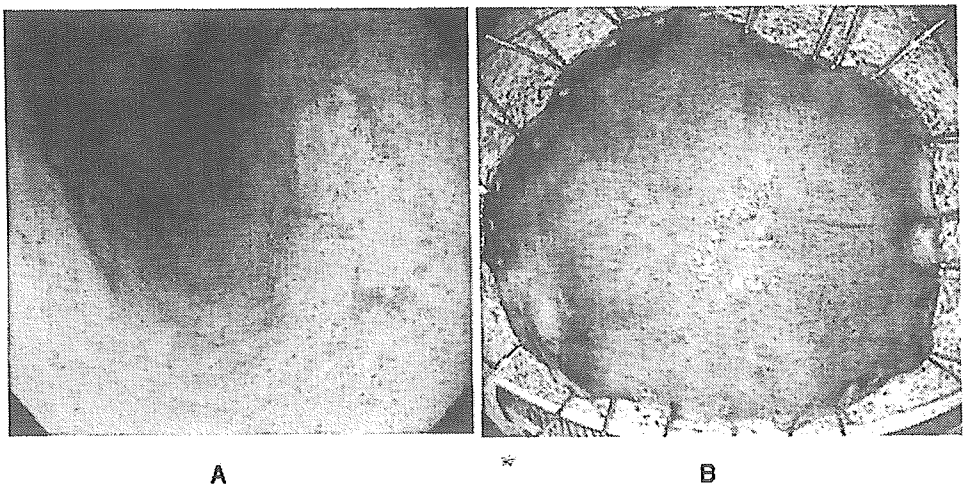
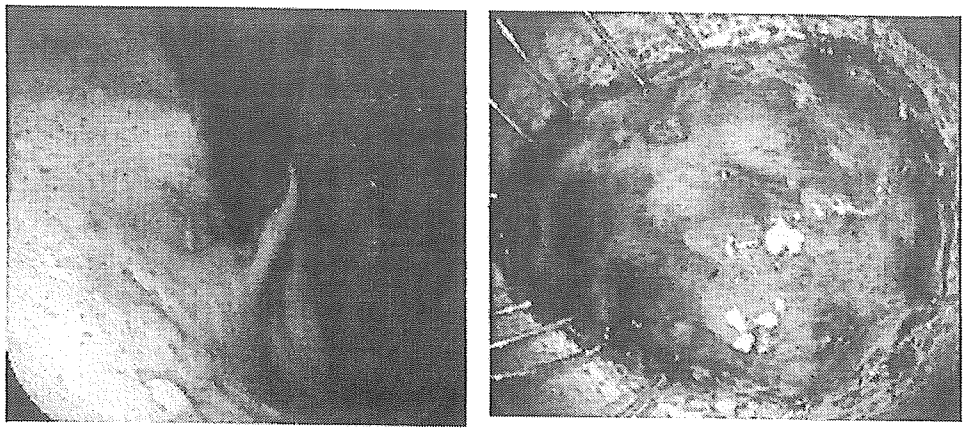
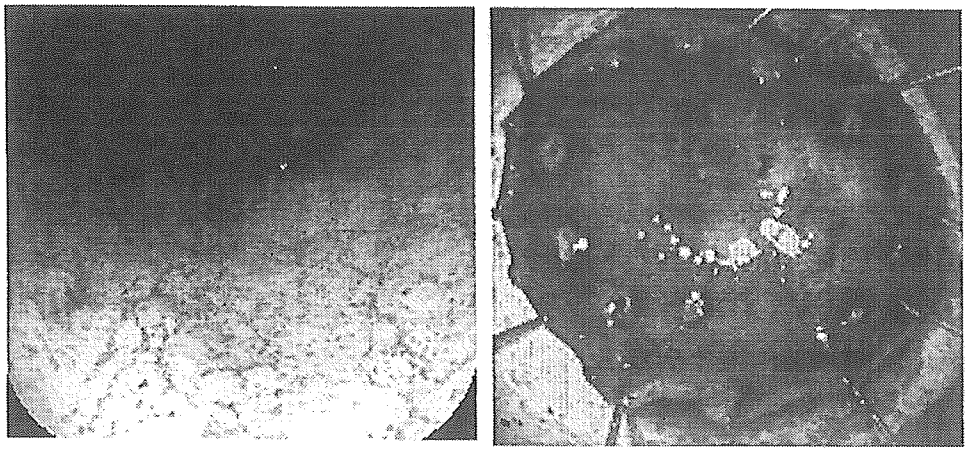


Figure 2. Case 1. A. Mucosal cancer with IIc+III type, 27 mm in size, located in the lesser curve of the middle gastric body. B. Resected specimen by ESD.



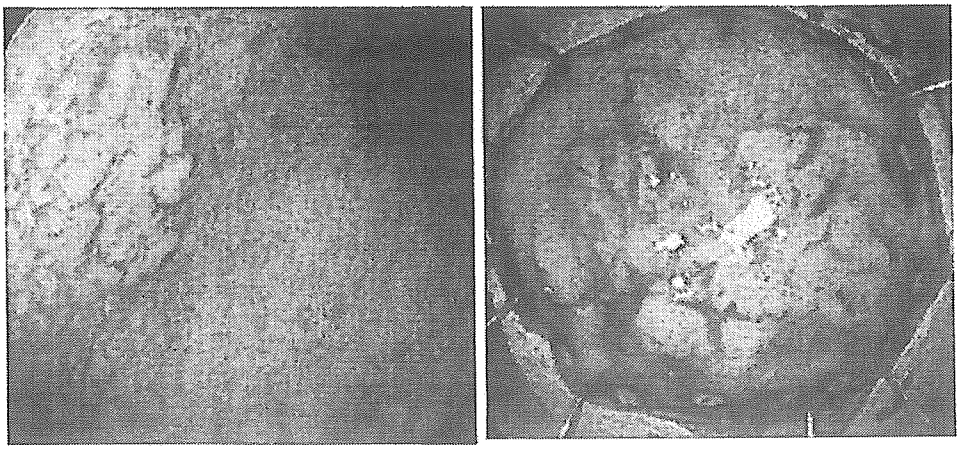
A B

Figure 3. Case 2. A. Mucosal cancer with IIc type, 10 mm in size, located in the lesser curve of the upper gastric body. B. Resected specimen by ESD.



A B

Figure 4. Case 5. A. Mucosal cancer with IIb type, 3 mm in size, located in the lesser curve of the middle gastric body. B. Resected specimen by ESD.



A B

Figure 5. Case 6. A. Mucosal cancer with IIa type, 21 mm in size, located in the anterior wall of the lower gastric body. B. Resected specimen by ESD.