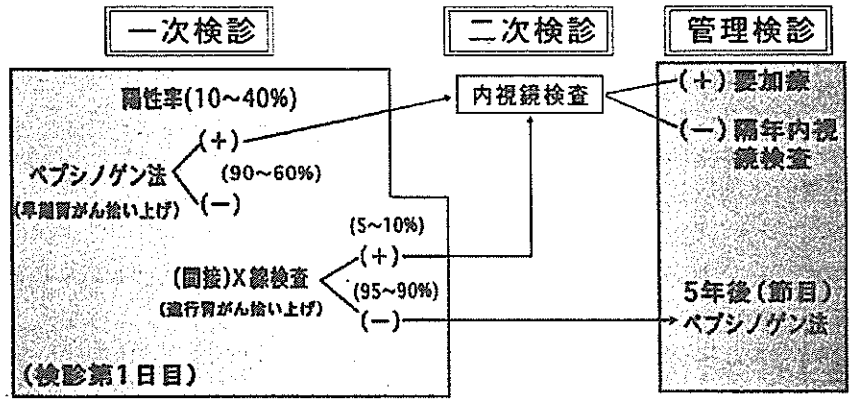

(厚生省がん研究助成金　三木班、2000)

図1　胃がん検診二段階（同日判定）法

内視鏡検査を実施するという方法が考えられる[11]．厚生労働省三木班が，PG法による胃がん検診を実施している5,000人規模の職域集団を1－5年間にわたり追跡を行ったところ，全対象者におけるPG陽性者（995例）の陰性者（4,173例）に対する胃がん発生の相対危険度は6.05（95%信頼区間（CI）1.80-20.30），男性のPG陽性者（865例）の陰性者（3,494例）に対する胃がん発生の相対危険度は8.34（95%CI 2.18-31.87）であった[2]．

ヘリコバクター検査との併用による胃がんスクリーニングの可能性

血清PG値に血清HpIgG抗体価検査を併用し，同時に胃内視鏡検査を行った人間ドック受診者の検診実施翌年以降の胃がん発見頻度を比較したところ，PG法陽性者からの胃がん発見率は有意に高く，反対にPG法陰性かつHp抗体陰性の者からは胃がん発見が1例もなく，胃がんローリスク群（低危険群）といえることがわかった[2]．血清PG値と血清Hp抗体価の組み合わせによって胃がんのハイリスク群を集約し，またローリスク群を設定することで，効果的に内視鏡検診を実施する方法を検討できる可能性がある[13][14]．

ペプシノゲン法の有効性評価

2001年3月に公表されたわが国におけるがん検診の有効性に関する評価報告書において，PG法は，胃がん死亡率減少効果に関する研究がなされていないため，評価を保留されている．厚生労働省三木班ではPG法の胃がん死亡率減少効果を証明すべく研究を進めている．PG法による胃がん検診を節目検診の際に受診した約5,500人を受診日から5年間追跡し，基準人口を日本全体として胃がん死亡の標準化死亡比（SMR）を算出した．胃がんのSMRは0.3を若干超える値であり，SMRの95%信頼区間は，1を含まないで1未満に分布しており，全国の胃がん死亡状況と比較して統計学的に有意に胃がん死亡率が低下していた．自己選択バイアス(self-selection bias）の影響は否定できないが，PG法による胃がん検診の胃がん死亡率減少効果を示唆する結果であった[15]．

平成16年度厚生労働省三木班研究成果

1）胃がん患者と性・年齢・人種をマッチさせた同一地域住民対照で①血清PGⅠ，Ⅱ値②血清抗HpIgG抗体価（HpAb）③血清抗Hp CagA蛋白抗体価（CagA）を測定し，胃がん罹患オッズ比を検討したところ，血清HpAbとCagA陽性で，かつPGI低値の組み合わせは，HpAbとCagAの両者が陰性で，かつPGⅠが正常の場合よりも41倍未分化型胃がんのリスクを高めていた[16][17]（表2）．

2）健常男性4,655人のコホートを10年間追跡した結果，胃がん発生がすべてHp感染陽性者から生じており，慢性胃炎進展にともなう胃がん発生のリスクの上昇があり，とくに化生性胃炎で年率1.25%であった[4][17]（図2）．

3）人間ドックで直接胃X線検査とPG法を同時に受診した9,993人を地域がん登録により1年間追跡した．直接胃X線検査の胃がん診断の感度は55.6%，特異度は93.8%，陽性反応適中度は1.6%，要精検率は6.3%であった．同様に，基準値（カットオフ値：PGⅠ70ng/ml以下かつPGⅠ／Ⅱ3.0以下）を要精検の判定基準とした場合のPG法の胃がん診断の感度は61.1%，特異度は85.3%，陽性反応適中度は0.7%，要精検率は14.8%であった[17]．

4）症例対照研究の手法によりPG法実施自治体におけ

表2　血清抗 *H.pylori* IgG 抗体価・CagA 蛋白抗体価・ペプシノゲン値3者組み合わせた胃がんオッズ比の検討

－*H.pylori* IgG抗体価・CagA蛋白抗体価・ペプシノゲン(PG)値の3者－

		全胃がん	分化型	未分化型
H.p.（－）・CagA（－）・PG I	正常	1	1	1
H.p.（－）・CagA（－）・PG I	低値	5.40※	5.06※	8.92
H.p.（＋）か CagA（＋）・PG I	正常	4.86※	3.64※	14.84※
H.p.（＋）か CagA（＋）・PG I	低値	9.21※	6.91※	40.74※
H.p.（－）・CagA（－）・PG I/II	正常	1	1	1
H.p.（－）・CagA（－）・PG I/II	低値	4.22※	3.54※	8.25
H.p.（＋）か CagA（＋）・PG I/II	正常	3.77※	2.57※	15.05※
H.p.（＋）か CagA（＋）・PG I/II	低値	6.88※	5.78※	12.58※

PG I 正常：≧30ng/ml，PG I/II 正常：≧2.0，※：$p<0.01$

(Nomura AMY, Miki K et al：J Infect Dis 191：2075－2781, 2005)

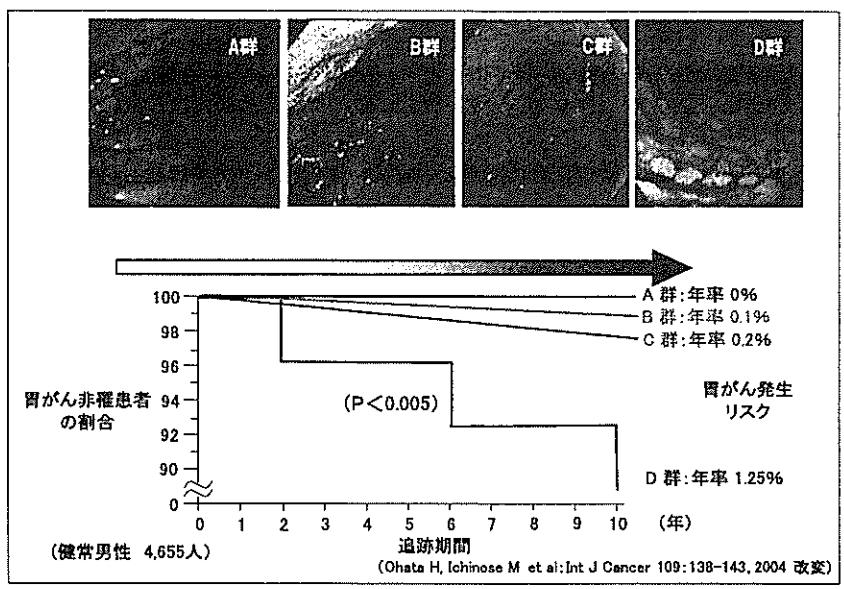

図2　*H.pylori* 感染（慢性萎縮性胃炎）の進展にともなう胃がん発生

るPG法受診の胃がん死亡減少効果を評価した．胃がん死亡症例41名のそれぞれに対して，同性で年齢±3歳の同じ地域在住者3名を対照とした．過去1年未満のPG法受診歴は，症例41名中0名で，対照では123名中23名（18.7%）の受診率であった（$p=0.0012$）．過去2年未満のPG法受診歴は，症例41名中2名（4.9%），対照123名中37名（30.1%）で，過去2年未満の受診オッズ比（95%信頼区間）は0.119（0.027－0.520）と有意に胃がん死亡の減少効果を認めた[17]．

ま と め

著者の考えている胃がん検（健）診の近未来像（図3）を提示した．胃がん検診の一次スクリーニングは検体検査になり，二次スクリーニングが画像診断となる．この画像診断には内視鏡検査だけでなくX線（間接・直接）検査もあり，受診者のニーズおよび2次精検者のマンパワーに合わせて使い分けるという方策である．しかし，現在，ただちに胃がん検診現場で実行しうる方策としての胃がん検診方式としては，DDW2005神戸の学会期間

中（2005. 9 .22）に開催された，第2回学会胃がん検診精度管理委員会（委員長今村清子理事）に著者が答申した意見書（表3）のとおりである．なお主文は厚労省研究班（三木班）2000年度報告書として既に報告したもの[10][11][18]である．今年度から新たに学会に設置された胃がん検診方式検討委員会で速やかに採択され，本学会推奨の方式（基準）となることを切望している．

文　献

1）厚生省がん研究助成金による「血清ペプシノゲン値による胃がんスクリーニングに関する研究（9－8）平成9－12年度研究報告」（主任研究者　三木一正）2001年10月

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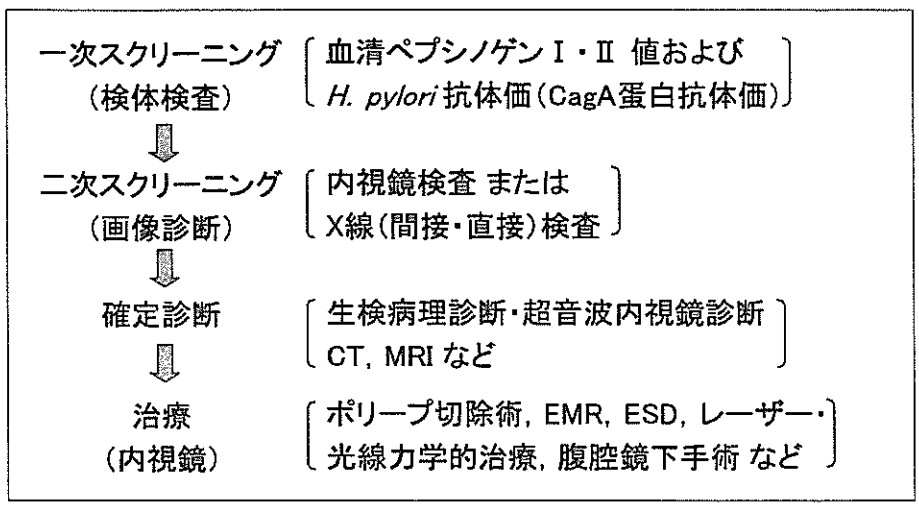

図3　胃がん検（健）診の近未来

表3　胃がん検診精度管理委員会 答申意見

（主文）　胃がん検診方式は，ペプシノゲン（PG）法とX線（P）法併用法の一次検診とすることが望ましい．二次検診は内視鏡検査とする．

（付）　1．PG（＋）のカットオフ値は基準値（PGI70ng/ml以下かつⅠ/Ⅱ比3.0以下）とする．
2．PG（－）（受診者の約70%）だけにXP（直接・間接いずれでも可）を検診第1日目に施行する．（二段階同日判定法）
3．XP撮影法は新・胃X線撮影法（直接・間接）ガイドラインに準拠する．

（副文）　1．ヘリコバクターピロリ（Hp）法を一次検診に用いる場合はPG（－），Hp（－）のA群（約30%）を二次検診から除くために使用する相応の根拠がある．
〔将来，PG法・Hp法併用法一次検診が推奨される可能性はあるが，現時点では，Hp測定方法（UBTで統一するなど）や陽性者の二次検診方法など検証を要する問題点が多く今後の継続検討課題である．〕
2．サイトカイン遺伝子多型（IL-10やTNF-α等），CagA蛋白等の遺伝子マーカーの応用は現在，研究課題である．今後の研究進展を注視する．
3．内視鏡単独検診は胃内視鏡検診標準化委員会の検討結果を参考にして推奨検診方式を呈示する．

（2005年9月22日）

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High Levels of Aberrant DNA Methylation in *Helicobacter pylori*–Infected Gastric Mucosae and its Possible Association with Gastric Cancer Risk

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Abstract **Introduction:** Risk prediction of gastric cancers is important to implement appropriate screening procedures. Although aberrant DNA methylation is deeply involved in gastric carcinogenesis, its induction by *Helicobacter pylori*, a strong gastric carcinogen, is unclear. Here, we analyzed the effect of *H. pylori* infection on the quantity of methylated DNA molecules in noncancerous gastric mucosae and examined its association with gastric cancer risk.

Experimental Design: Gastric mucosae were collected from 154 healthy volunteers (56 *H. pylori* negative and 98 *H. pylori* positive) and 72 cases with differentiated-type gastric cancers (29 *H. pylori* negative and 43 *H. pylori* positive) by endoscopy. The numbers of DNA molecules methylated and unmethylated for eight regions of seven CpG islands (CGI) were quantified by quantitative PCR after bisulfite modification, and fractions of methylated molecules (methylation levels) were calculated.

Results: Among healthy volunteers, methylation levels of all the eight regions were 5.4- to 303-fold higher in *H. pylori* positives than in *H. pylori* negatives ($P < 0.0001$). Methylation levels of the *LOX*, *HAND1*, and *THBD* promoter CGIs and *p41ARC* exonic CGI were as high as 7.4% or more in *H. pylori*–positive individuals. Among *H. pylori*–negative individuals, methylation levels of all the eight regions were 2.2- to 32-fold higher in gastric cancer cases than in age-matched healthy volunteers ($P \leq 0.01$). Among *H. pylori*–positive individuals, methylation levels were highly variable, and that of only *HAND1* was significantly increased in gastric cancer cases (1.4-fold, $P = 0.02$).

Conclusions: It was indicated that *H. pylori* infection potently induces methylation of CGIs to various degrees. Methylation levels of specific CGIs seemed to reflect gastric cancer risk in *H. pylori*–negative individuals.

Gastric cancer is one of the most common malignancies worldwide and remains a leading cause of cancer death in Asia and some European countries (1). To reduce its mortality, early detection by endoscopy and curative resection are important (2). However, considering the potential risk and costs of early detection by endoscopic examination, implementation reflecting an individual's risk for developing a gastric cancer would be ideal. Also, endoscopic mucosal resection, which conserves the noncancerous gastric mucosae, is becoming popular, and the problem of metachronous gastric cancer recurrence is being recognized (3). Again, if the future risk of developing metachronous cancers in a specific case can be estimated, the information will be useful in the decision on either surgical resection or endoscopic mucosal resection for the case.

The major etiologic risk factor for gastric cancers is *Helicobacter pylori* infection, which increases gastric cancer risk 2.2- to 21-fold (4–6). In an animal model with Mongolian gerbil chronic infection with *H. pylori* rarely induces gastric cancers by itself, but markedly enhances their incidences after initiation with a mutagen, such as *N*-methyl-*N*-nitrosourea (7). This promoting effect of *H. pylori* has been attributed to the induction of chronic inflammation and cell proliferation (8). Cell proliferation increases a chance for initiated cells to escape growth suppression and undergo further mutations. Other risk factors for gastric cancers include high salt intake and smoking (9), and a cancer risk marker incorporating these factors is awaited.

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As an additional mechanism of gastric cancer induction by *H. pylori* infection, induction of aberrant methylation was suggested by Chan et al. (10). They observed that *E-cadherin* methylation was more frequent in the gastric mucosae of dyspepsia cases with *H. pylori* infection than those without. In contrast, Kang et al. (11) did not observe a difference in the number of methylated genes in gastric mucosae with and without *H. pylori* infection. The discrepancy could be due to the lack of quantification of aberrantly methylated DNA molecules. Aberrant methylation in noncancerous tissues occurs only in a fraction of cells, which is expected to be highly variable, and qualitative analysis of methylation does not seem suitable. Also, different CpG islands (CGI) and, even within a CGI, different regions show different susceptibility to aberrant DNA methylation (12), and analysis considering the different susceptibility has not been done. Most importantly, there is no former study regarding the relationship between the level of aberrant methylation in gastric mucosae and risks of gastric cancer development. It seems possible that levels of aberrant methylation could reflect past exposure to *H. pylori* and other carcinogens, and that the methylation levels could be used as a cancer risk marker.

In this study, we aimed to clarify the effects of *H. pylori* infection on the induction of aberrant methylation by quantifying methylation levels of multiple CGIs and regions in healthy volunteers with and without *H. pylori* infection. Then, to clarify whether accumulated levels of aberrant DNA methylation are associated with a risk of gastric cancer development, we quantified methylation levels in gastric mucosae of healthy volunteers and noncancerous gastric mucosae of gastric cancer cases, which are known to have an elevated risk of gastric cancers (13, 14).

Materials and Methods

Cases, tissue samples, and DNA extraction. Healthy volunteers (82 males and 72 females) with an average age of 54.2 (range, 23-98) were recruited with informed consents on the occasion of a gastric cancer screening program under the approval of institutional review boards. Cases with well-differentiated gastric cancers (60 males and 12 females) with an average age of 67.2 (range, 37-85) were recruited with informed consents and under the approval of institutional review boards. To obtain a group of healthy volunteers whose average age was matched to the cancer cases, the same number of volunteers as cancer cases was randomly selected from each age group. The age-matched group (35 males and 37 females) had an average age of 64.4 (range 39-91; Table 1). *H. pylori* infection status was analyzed by a serum anti-*H. pylori* IgG antibody test (SBS, Kanagawa, Japan), rapid urease test (Otsuka, Tokushima, Japan), or culture test (Eiken, Tokyo, Japan). The sensitivities of the serum anti-*H. pylori* IgG antibody test and rapid urease test are ≥90% of the culture test (15, 16).

Gastric mucosae were obtained by endoscopic biopsy of two standard sites, the upper corpus and antral regions in the lessor curvature, with sterilized biopsy forceps (Olympus, Tokyo, Japan). Gastric cancer cases that had cancers in either of the two standard sites were excluded from the analysis. Histologic analysis of selected biopsied materials showed that these samples contain 40% to 80% of epithelial tissues. The samples were frozen and stored at −80°C. High molecular weight DNA was extracted by the phenol/chloroform method.

Sodium bisulfite modification and quantitative methylation-specific PCR. Bisulfite treatment was done using 500 ng genomic DNA, digested with *Bam*HI, as previously described (17), and the treated DNA was suspended in 40 μL of TE buffer. An aliquot of 2 μL was used for real-time PCR with a primer set specific to methylated or unmethylated sequences. Using DNA from gastric mucosae from a young individual without *H. pylori* infection and DNA methylated with *Sss*I methylase (New England Biolabs, Beverly, MA), an annealing temperature specific for a primer set was determined. Real-time PCR was done using SYBR Green PCR Core Reagents (PE Biosystems, Warrington, United Kingdom) and an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Standard DNA was prepared by cloning PCR products into the pGEM-T Easy vector (Promega, Madison, WI). The number of molecules in a test sample was determined by comparing its amplification with those of samples containing a known number of molecules (10-10^5 molecules). The number of molecules methylated and unmethylated for a genomic region in a sample was measured separately, and the methylation level was calculated as the fraction of methylated molecules in the total number of DNA molecules (number of methylated molecules + number of unmethylated molecules). The primer sequences and PCR conditions are shown in Supplementary Table S1, and standard DNA for real-time methylation-specific PCR is available upon request.

Statistical analysis. The differences of mean methylation levels were analyzed by using the *t* test Welch method (both sided). Association between the age and methylation level was analyzed by calculating correlation coefficients and t_0 values.

Results

Quantification of methylation levels and reproducibility. We analyzed methylation levels of two regions of the *p16* promoter CGI and one region of six genes (*LOX, FLNc, HRASLS, HAND1, THBD*, and *p41ARC*; Fig. 1A), which could be methylated in human gastric cancers (18, 19). The two regions of *p16* promoter CGIs were selected because one region (core region)

Table 1. Characteristics of the individuals analyzed

	All cases		Age matched	
	n (male:female)	Mean age (range)	*n* (male:female)	Mean age (range)
Healthy volunteers				
HP (−)	56 (30:26)	51 (25-91)	29 (14:15)	63 (48-91)
HP (+)	98 (52:46)	56 (23-98)	43 (21:22)	64 (39-86)
Gastric cancer cases				
HP (−)	29 (24:5)	69 (52-85)	n/a	n/a
HP (+)	43 (36:7)	67 (37-85)	n/a	n/a

Abbreviations: *HP* (−), *H. pylori* negative; *HP* (+), *H. pylori* positive; n/a, not applicable.

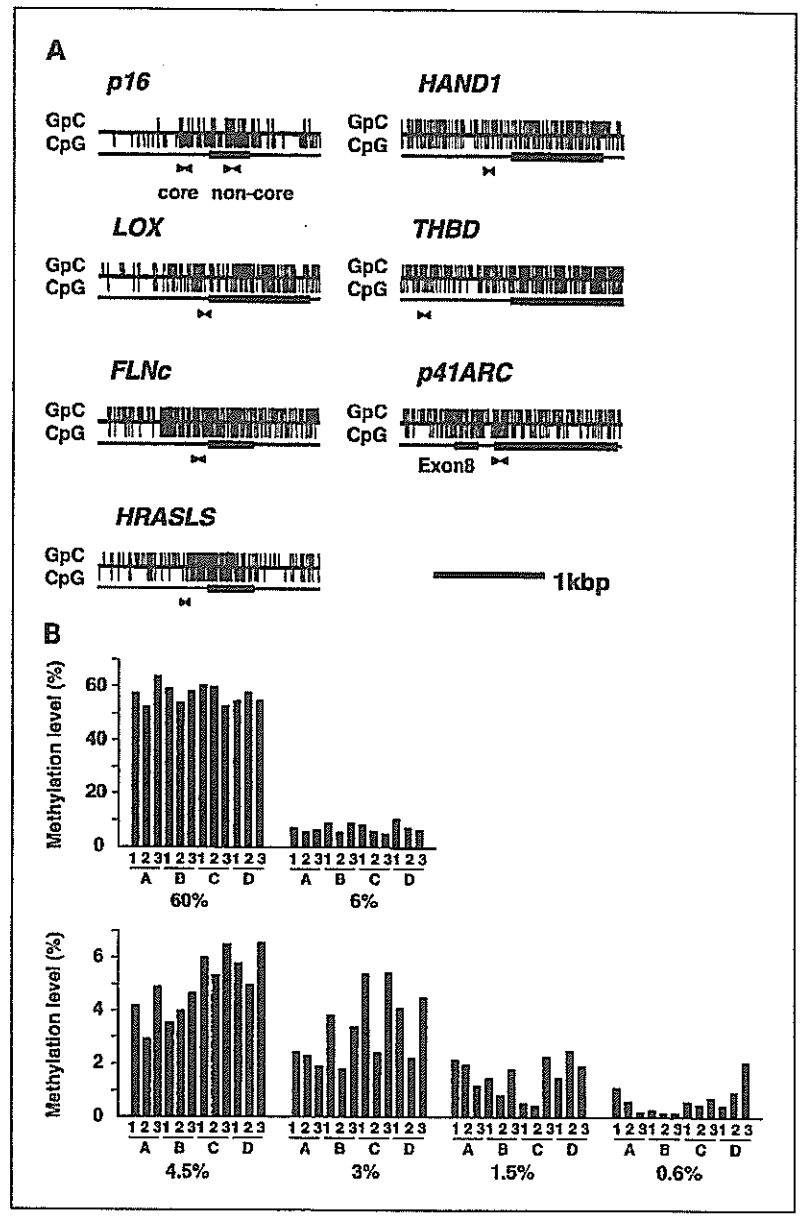

Fig. 1. Locations of regions analyzed and reproducibility of methylation levels. *A*, CGIs and regions analyzed. *B*, reproducibility of methylation levels. Two independent bisulfite modifications (*A* and *B*; *C* and *D*) were done and four independent quantitative PCRs were done in triplicate. The methylation levels obtained were highly reproducible.

was known to be critical for *p16* silencing but resistant to methylation and the other region (noncore region) was known to be susceptible to methylation but does not suppress gene expression (12). For *LOX*, *FLNc*, *HRASLS*, and *HAND1*, core regions of their promoter CGIs were analyzed. For *THBD*, noncore region of its promoter CGI was analyzed because the core region was not methylated at all (data not shown). For *p41ARC*, we analyzed its exonic CGI, which was frequently methylated in gastric cancers (19).

The reproducibility of the values obtained by quantitative methylation-specific PCR was analyzed for the *HAND1* CGI. Test DNA samples were prepared by mixing DNA completely methylated by *Sss*I methylase and that in which no methylation was detected at the ratios of 60%, 6%, 4.5%, 3%, 1.5%, and 0.7% of methylation. The same DNA was modified by bisulfite on two different dates (groups A and B on the same date, and groups C and D on the other date). Each group was analyzed by quantitative PCR on different dates in triplicate. The resultant 12 values (mean ± SD) for the three samples were as follows: 57.1 ± 3.5%, 7.3 ± 1.3%, 5.0 ± 1.2%, 3.3 ± 1.3%, 1.6 ± 0.7%, and 0.7 ± 0.5% (Fig. 1B). Therefore, methylation levels obtained here were highly reliable when they were larger than 1.5% to 3%, and were also reliable but more number of measures were necessary when smaller than 1.5% to 3%.

***High levels of aberrant methylation in gastric mucosae with* H. pylori.** Methylation levels of the eight regions were analyzed by quantitative methylation-specific PCR in 56 *H. pylori*–negative volunteers and 98 *H. pylori*–positive volunteers (Fig. 2; Table 2). For all the eight CGIs analyzed, methylation levels in the *H. pylori*–positive healthy volunteers were significantly and markedly elevated compared with those in the *H. pylori*–negative healthy volunteers. In the corpus, the mean methylation levels were elevated 303-fold (*p16* core region), 20-fold (*p16* noncore region), 14-fold (*LOX*), 11-fold (*THBD*), 49-fold (*FLNc*), 13-fold (*HRASLS*), 9.3-fold (*HAND1*), and 5.4-fold (*p41ARC*). In the antrum, they were

elevated 54-fold (*p16* core region), 22-fold (*p16* noncore region), 16-fold (*LOX*), 17-fold (*THBD*), 30-fold (*FLNc*), 18-fold (*HRASLS*), 7.8-fold (*HAND1*), and 5.7-fold (*p41ARC*). Especially, methylation levels of *LOX*, *HAND1*, *THBD*, and *p41ARC* were higher than 7.4% in *H. pylori*–positive individuals, and this unequivocal effect of *H. pylori* infection supported the increases in methylation levels of the other CGIs in smaller ranges. This strongly indicated that *H. pylori* infection potently induced aberrant methylation in multiple CGIs. Mean methylation levels were in the same range in the corpus and antrum.

Effect of age and sex on methylation levels. Because age-dependent methylation was reported for various CGIs (20, 21), association between age and methylation levels of antral mucosa was analyzed by calculating correlation coefficients. In the 56 *H. pylori*–negative healthy volunteers, correlation coefficients (*P* values) for *p16* core region, *p16* noncore region, *LOX*, *THBD*, *FLNc*, *HRASLS*, *HAND1*, and *p41ARC* were 0.09 (0.51), 0.23 (0.08), 0.18 (0.18), 0.26 (0.05), 0.17 (0.21), 0.34 (0.01), 0.32 (0.02), and 0.29 (0.03), respectively. In the 98 *H. pylori*–positive healthy volunteers, they were 0.18 (0.08), 0.13 (0.20), −0.02 (0.85), 0.08 (0.43), −0.07 (0.49), 0.05 (0.62), 0.18 (0.08), and 0.13 (0.20), respectively. Only *HRASLS*, *HAND1*, and *p41ARC* showed very weak correlation ($0.01 < P < 0.05$) with age in *H. pylori*–negative healthy volunteers.

Because males have a twice as high incidence of gastric cancers as females (2), we also examined the sex differences of methylation levels. However, no significant differences were observed between the 30 males and 26 females among the 56 *H. pylori*–negative healthy volunteers, or between the 52 males and 46 females among the 98 *H. pylori*–positive healthy volunteers.

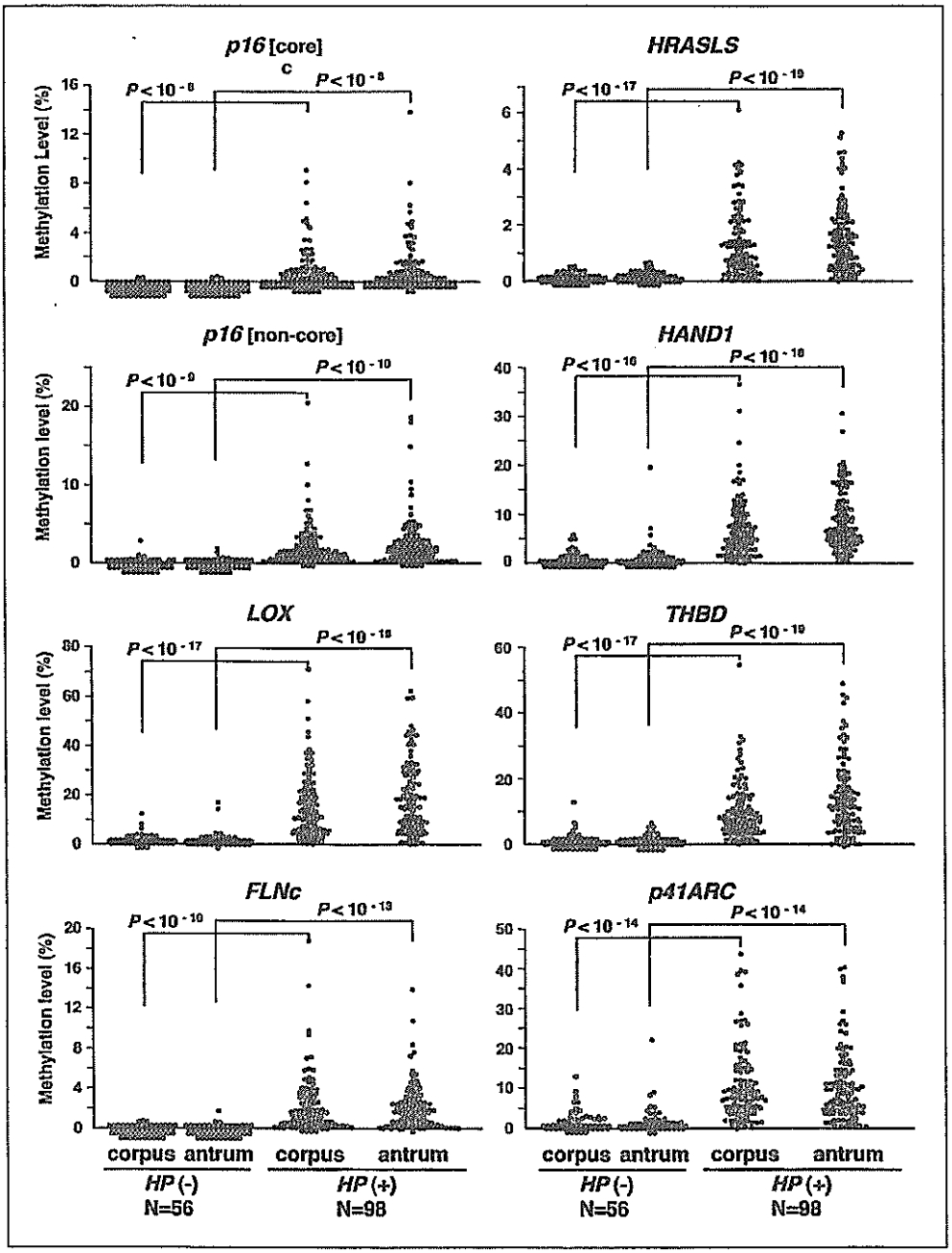

Fig. 2. Higher levels of methylation in gastric mucosae of *H. pylori*–positive volunteers than in those of *H. pylori*–negative volunteers. Methylation levels were measured in the corpus and antrum of 56 *H. pylori*–negative volunteers and 98 *H. pylori*–positive volunteers. All the eight CGIs (core region of *p16*, noncore regions of *p16* and *THBD*; core regions of *LOX*, *HRASLS*, *FLNc*, and *HAND1*; and *p41ARC* exonic CGI) showed significantly elevated methylation levels (5.4- to 303-fold) in the *H. pylori*–positive volunteers. Methylation levels in the corpus were at the same levels as those in the antrum.

Table 2. Methylation levels of the seven CGIs in *H. pylori*–negative and *H. pylori*–positive individuals and in healthy volunteers and gastric cancer cases

	n	Mean age	Corpus							Antrum						
			p16 (core)	*p16* (non)	*LOX*	*FLNc*	*HRASLS*	*HAND1*	*THBD*	*p41ARC*	*p16* (core)	*p16* (non)	*LOX*	*FLNc*	*HRASLS*	*HAND1*	*THBD*	*p41ARC*
HV																		
HP(−)	56	51	0.0%	0.1%	1.2%	0.0%	0.1%	0.8%	1.0%	2.1%	0.0%	0.1%	1.2%	0.1%	0.1%	1.1%	0.8%	1.9%
HP(+)	98	57	0.9%	2.2%	16.0%	2.3%	1.3%	7.4%	10.7%	11.2%	1.1%	2.6%	18.8%	2.2%	1.5%	8.8%	14.2%	10.7%
P			$<10^{-8}$	$<10^{-9}$	$<10^{-17}$	$<10^{-10}$	$<10^{-17}$	$<10^{-16}$	$<10^{-17}$	$<10^{-14}$	$<10^{-8}$	$<10^{-10}$	$<10^{-19}$	$<10^{-13}$	$<10^{-19}$	$<10^{-18}$	$<10^{-19}$	$<10^{-14}$
GCC																		
HP(−)	29	69	0.2%	0.6%	8.2%	0.6%	0.6%	4.1%	7.1%	6.6%	0.2%	0.5%	4.6%	1.3%	0.4%	7.5%	5.5%	4.9%
HP(+)	43	67	0.4%	2.0%	12.7%	0.8%	1.2%	8.1%	12.3%	11.5%	0.3%	2.5%	11.2%	1.0%	0.8%	11.3%	10.2%	7.8%
P			0.07	<0.01	0.02	0.17	<0.01	<0.01	0.01	<0.01	0.28	0.04	<0.01	0.35	0.02	0.05	0.02	0.02
HP(−)																		
AM-HV	29	63	0.0%	0.2%	1.7%	0.1%	0.1%	1.0%	1.3%	3.1%	0.0%	0.2%	1.4%	0.1%	0.1%	1.7%	1.1%	2.2%
GCC	29	69	0.2%	0.6%	8.2%	0.6%	0.6%	4.1%	7.1%	6.6%	0.2%	0.5%	4.6%	1.3%	0.4%	7.5%	5.5%	4.9%
P			<0.01	0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.05	0.02	0.02	0.03	<0.01	<0.01	<0.01	0.02
HP(+)																		
AM-HV	43	64	1.0%	1.9%	12.8%	1.4%	1.2%	6.7%	9.4%	9.4%	0.8%	2.1%	13.9%	1.5%	1.2%	7.8%	10.0%	9.8%
GCC	43	67	0.4%	2.0%	12.7%	0.8%	1.2%	8.1%	12.3%	11.5%	0.3%	2.5%	11.2%	1.0%	0.8%	11.3%	10.3%	7.8%
P			0.03	0.44	0.48	0.02	0.48	0.13	0.07	0.11	0.07	0.38	0.17	0.09	0.05	0.02	0.45	0.12

Abbreviations: HV, healthy volunteers; GCC, gastric cancer cases (noncancerous mucosae); AM-HV, age-matched healthy volunteers; non, noncore.

Association between high methylation levels and gastric cancer development. Finally, we examined whether accumulated levels of aberrant DNA methylation in gastric mucosae are associated with a risk of gastric cancer. Methylation levels in noncancerous gastric mucosae of 72 gastric cancer cases (29 *H. pylori*–negative and 43 *H. pylori*–positive cases) were compared with those of 72 healthy volunteers (29 *H. pylori*–negative and 43 *H. pylori*–positive individuals) that were randomly selected to match the average age and *H. pylori* infection status of gastric cancer cases (Table 2).

When *H. pylori*–negative healthy volunteers and *H. pylori*–negative gastric cancer cases were compared, mean methylation levels of antral mucosae in gastric cancer cases (Fig. 3) were significantly elevated at 4.9-fold (*p16* core region), 2.3-fold (*p16* noncore region), 3.3-fold (*LOX*), 5.1-fold (*THBD*), 10-fold (*FLNc*), 3.9-fold (*HRASLS*), 4.4-fold (*HAND1*), and 2.2-fold (*p41ARC*). The same tendency was observed in the corpus. When *H. pylori*–positive healthy volunteers and *H. pylori*–positive gastric cancer cases were compared, variations within both groups were very large. A possibly significant increase ($P = 0.02$) was observed only for *HAND1* at 1.4-fold.

Discussion

It was shown here that significantly higher levels of aberrant methylation (5.4- to 303-fold) were present in multiple CGIs in the gastric mucosae of healthy volunteers with *H. pylori* infection. This finding strongly indicated that *H. pylori* infection potently induces aberrant methylation in multiple CGIs, although there has been controversy (10, 11). The induction of aberrant methylation by the strong gastric carcinogen *H. pylori* is in a good agreement with the fact that tumor suppressor genes, like *p16*, *E-cadherin*, and *hMLH1*, are inactivated more frequently by aberrant DNA methylation than by mutations in gastric cancers (2). Aberrant DNA methylation was shown to be present in noncancerous mucosae of ulcerative colitis by pioneering studies (21, 22), and a role of chronic inflammation in methylation induction has been proposed. Because *H. pylori* infection also causes strong chronic inflammation (23), the role of chronic inflammation in induction of aberrant methylation seems very clear.

Methylation levels of all of the eight CGIs were associated with gastric cancer risk in *H. pylori*–negative individuals. It must be noted that clinical tests for *H. pylori* infection detect only current (culture and urease tests) or recent (serum antibody test) status of *H. pylori* infection and cannot detect past exposure to *H. pylori* (4, 16). On the other hand, epidemiologic studies showed that past exposure to *H. pylori*, rather than current exposure, is more closely associated with a risk of gastric cancer development and that a majority of *H. pylori*–negative gastric cancer cases had past exposure to *H. pylori* (5, 24).

However, methylation levels in *H. pylori*–positive individuals were higher than *H. pylori*–negative gastric cancer cases. The high methylation levels in the *H. pylori*–positive individuals are considered to drop down to various degrees when active *H. pylori* infection discontinues as observed in the *H. pylori*–negative gastric cancer cases. This suggests that *H. pylori* infection induces DNA methylation in both stem cells, which will persist, and nonstem cells, which will drop off the gastric mucosae in a few days (25). If the methylation status of stem cells is copied into the cells in the entire gland without active induction, the methylation levels in the gastric mucosae will reflect the fraction of methylated stem cells among the entire

stem cell population and thus a fraction of stem cells with increased cancer risk. It seems important to examine whether eradication of *H. pylori* leads to decrease in methylation levels.

Mechanistic analysis of how *H. pylori* infection induces aberrant DNA methylation is necessary. *H. pylori* infection almost always induces chronic inflammation and cell proliferation (8). Cell proliferation itself has been suggested as a promoting factor for *de novo* DNA methylation (21, 26). In addition, expression of many genes is repressed during the inflammatory processes and decreased gene expression is known to promote *de novo* methylation (27–29). Further, it was recently reported that stimulation of myeloma cells by interleukin-6 increased expression of DNA methyltransferase 1 (*DNMT1*) mRNA expression (30).

The methylation level of the *p16* core region was consistently much lower than methylation levels of other CGIs. No methylation was detected in 46 (47%) of 98 *H. pylori*–positive healthy volunteers, whereas it was only 3 (3%) when the *p16* noncore region was analyzed. Also, absolute levels of methylation were much higher in *LOX*, *HAND1*, *THBD*, and *p41ARC* of *H. pylori*–positive individuals and *H. pylori*–negative gastric cancer cases. This suggested that extensive methylation of multiple, and possibly preferential, CGIs precedes infrequent occurrence of methylation of a core region of a promoter CGI of critical tumor suppressor gene(s).

As for the effect of locations within the stomach, no significant difference in methylation levels was observed between the corpus and antrum, regardless of *H. pylori*

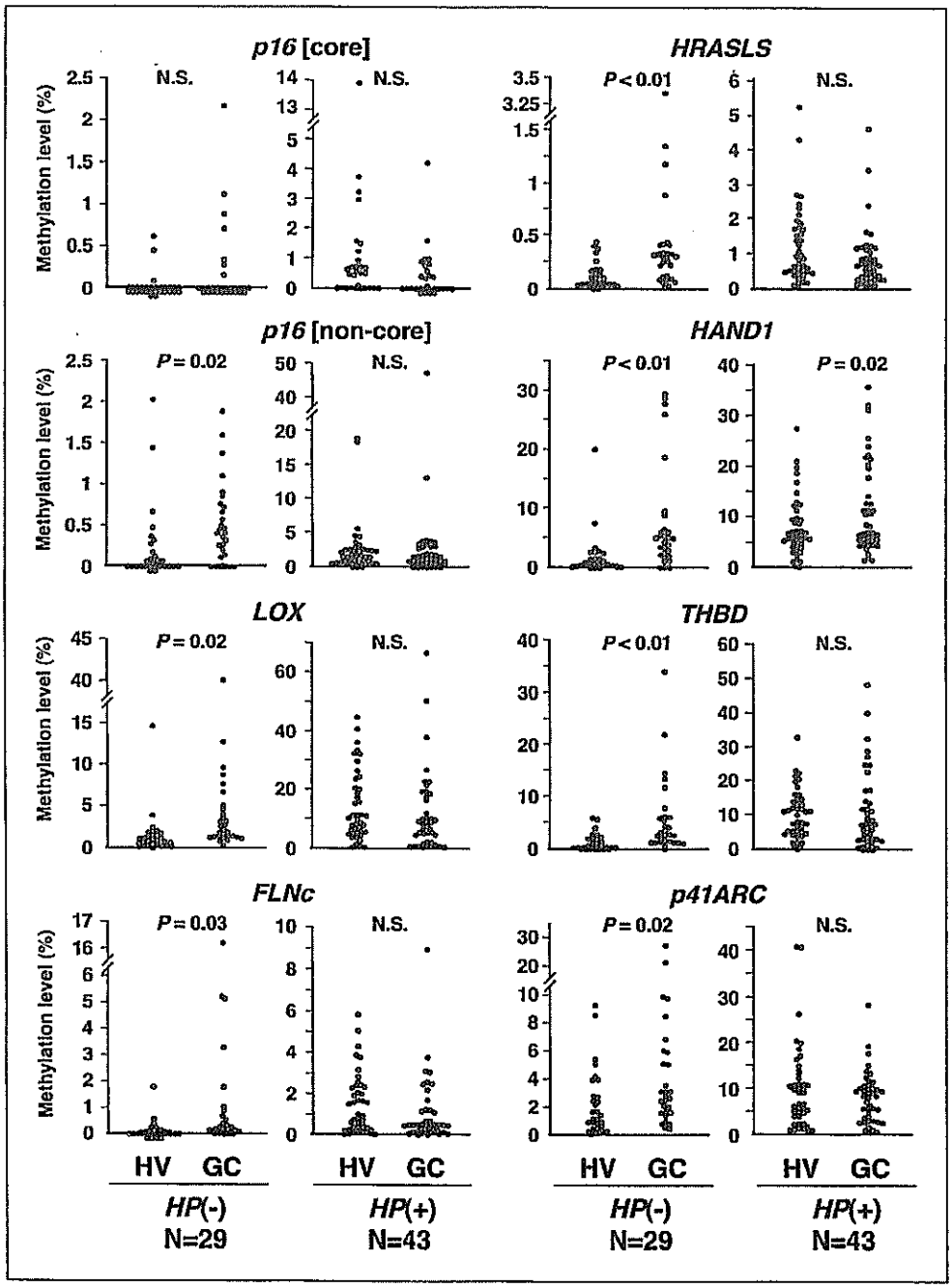

Fig. 3. Association between high methylation levels and a risk of gastric cancer development. Methylation levels of antral mucosae were measured in 29 *H. pylori*–negative and 43 *H. pylori*–positive cases with differentiated-type gastric cancers (*GC*), and the levels were compared with those in 29 *H. pylori* (*HP*)–negative and 43 *H. pylori*–positive age-matched healthy volunteers (*HV*). Among the *H. pylori*–negative individuals, methylation levels of gastric cancer cases were significantly higher (2.2- to 10-fold) than those in healthy volunteers, which showed that methylation levels in noncancerous gastric mucosae are associated with a risk of gastric cancer development. Among the *H. pylori*–positive individuals, methylation levels were highly variable within each group, and a significant increase was observed only for *HAND1* at 1.4-fold. N.S., not significant.

infection status. As for the effect of histologic changes, analysis of limited number of samples showed methylation levels of the eight CGIs were not associated with mucosal atrophy, intestinal metaplasia, or degree of inflammation (data not shown). It seems important to search for specific CGIs whose methylation levels are associated with *H. pylori* infection, with a gastric cancer risk, and with histologic changes, respectively, because various CGIs and regions within one CGI show different susceptibility to methylation (12).

In conclusion, it was indicated that *H. pylori* infection potently and temporarily induces methylation of multiple CGIs. Methylation levels of specific CGIs in noncancerous gastric mucosae may be associated with gastric cancer risk in *H. pylori*–negative individuals.

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ORIGINAL ARTICLES

Endoscopic Submucosal Dissection of Esophageal Squamous Cell Neoplasms

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Background & Aims: Endoscopic submucosal dissection (ESD) has recently been developed for en bloc resection of stomach neoplasms, which results in high tumor eradication rates as well as a modality for the precise histologic assessment of the entire lesion. Application of the technique is desirable for esophageal squamous cell neoplasms (SCNs), but there have been no reports on the use of this procedure in the esophagus. *Methods:* An ESD with methods similar to those used for resections of early gastric cancer was performed on 58 consecutive esophageal SCNs with preoperative diagnoses of intraepithelial neoplasm or intramucosal invasive carcinoma occurring in 43 enrolled patients. The therapeutic efficacy, complications, and follow-up results were assessed. *Results:* The rate of en bloc resection was 100% (58/58), and en bloc resection with tumor-free lateral/basal margins (R0 resection) was 78% (45/58). There was no evidence of significant bleeding. Perforation occurred in 4 (6.9%) patients during the ESD, who were managed by conservative medical treatments after endoscopic closure of the perforation. Removal of 9 (16%) lesions resulted in esophageal stricture requiring balloon dilation after ESD. Of 40 lesions occurring in 31 patients fulfilling the criteria of node-negative tumors (mean follow-up, 17 months), 1 lesion resected by en bloc resection with nonevaluable tumor-free lateral margins (Rx [lateral] resection) recurred locally 6 months after ESD, which was treated successfully by a second ESD procedure. *Conclusions:* The ESD is applicable to the esophagus with promising results, but notification of risk is essential.

With the recent development of endoscopy and iodine staining,[1,2] the discovery of esophageal squamous cell neoplasms (SCNs) indicated for local treatment has increased markedly. Endoscopic mucosal resection (EMR) has been aggressively indicated for select localized neoplasms as an alternative to esophagectomy, especially in Japan,[3] because the rates of surgical mortality and postsurgical complications related to esophagectomy are high.[4–6] The long-term survival outcomes after EMR in the esophagus show similar efficacy when compared with esophagectomy for small, early-stage neoplasms.[7,8]

A large number of retrospective histopathologic analyses of surgically resected esophageal SCNs showed that noninvasive carcinoma (carcinoma in situ, m1) and intramucosal invasive carcinoma limited to the lamina propria mucosae (m2) without vessel infiltration have no lymph node or distant metastases[3,9,10] and might be considered targets for endoscopic resection. However, large-sized or complex-shaped lesions might be treated by esophagectomy, chemotherapy, or radiation therapy because of technical difficulties in achieving complete removal of these lesions. By contrast, conventional EMR techniques such as the "inject, lift, and cut technique" or the "inject, suck, and cut technique" are limited in resection size, and large lesions have to be resected in multiple fragments. Moreover, the resected area cannot be precisely controlled by the operators, which might lead not only to incomplete removal of even small lesions but also to excessive non-neoplastic mucosal resection. Unnecessary excessive resection should be avoided, especially in the esophagus, so as not to cause deformity and stenosis of the narrow esophageal space. A newly developed therapeutic endoscopy with cutting knives, endoscopic submucosal dissection (ESD), was originally developed for en bloc resection of large and ulcerative

Abbreviations used in this paper: EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; SCN, squamous cell neoplasm

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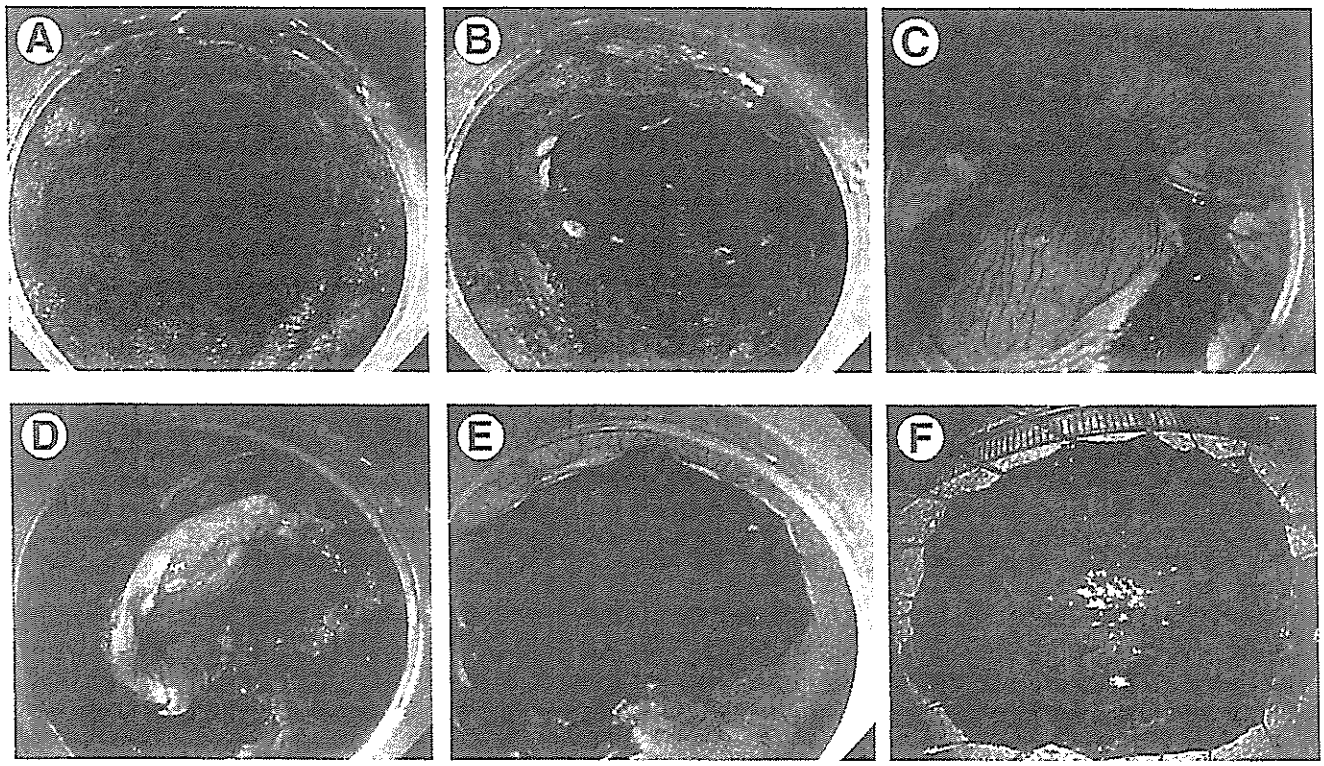

Figure 1. ESD for esophageal neoplasms. (*A*) Chromoendoscopy with iodine staining to demarcate the lesion from the non-neoplastic area. (*B*) Marking around the lesion. (*C*) Initial mucosal incision after submucosal injection at the distal margin of the lesion. (*D*) Mucosal incision after submucosal injection at the proximal margin of the lesion and subsequent submucosal dissection from the proximal end. (*E*) Artificial ulcer after removal. (*F*) Complete resection of the lesion in one piece.

neoplasms in the stomach,[11–14] and these techniques have been applied to other organs of the gastrointestinal tract including the esophagus and colorectum.[15–17] The outcomes of ESD have not been reported in detail, even in the stomach, because the long-term data are still being collected. Furthermore, few studies have elucidated the technical feasibility of the procedure in other gastrointestinal tract organs, including the esophagus and colorectum. In this study, we assessed ESD with our own technique for esophageal SCNs with special reference to the technical feasibility and short-term follow-up outcomes.

Patients and Methods

Fifty-eight consecutive superficial esophageal SCNs occurring in 43 patients were resected by ESD between January 2002 and September 2005 at the University of Tokyo Hospital, Tokyo, Japan. All patients with esophageal neoplasm who had a preoperative diagnosis of high-grade intraepithelial neoplasm (high-grade dysplasia and noninvasive carcinoma) or intramucosal invasive carcinoma were candidates for ESD. Diagnosis was made by using chromoendoscopy with iodine staining, endoscopic biopsy, and occasionally by endoscopic ultrasonography for suspicious lesions of submucosal invasion. All patients were informed of the risks and benefits of several treatment options including ESD, conventional EMR, ablation therapy, conventional surgery, and radiation therapy with or without concomitant chemotherapy, and written informed consent to perform ESD was obtained from all the patients preoperatively.

Endoscopic Submucosal Dissection Procedure

ESD was carried out by using a single-channel upper gastrointestinal endoscope with a water-jet system (XGIF-Q240M; Olympus Optical Co, Tokyo, Japan, or EG-2931; Pentax Co, Tokyo, Japan) and a high-frequency generator with an automatically controlled system (ENDOCUT mode) (Erbotom ICC 200; ERBE Elektromedizin GmbH, Tübingen, Germany). The transparent attachment was fitted on the tip of the endoscope mainly to obtain a constant endoscopic view and to create tension on the connective tissue for the submucosal dissection. A representative case of the procedure is shown in Figure 1.

Marking around the lesion. Lugol chromoendoscopy was necessary before marking the lateral margin of

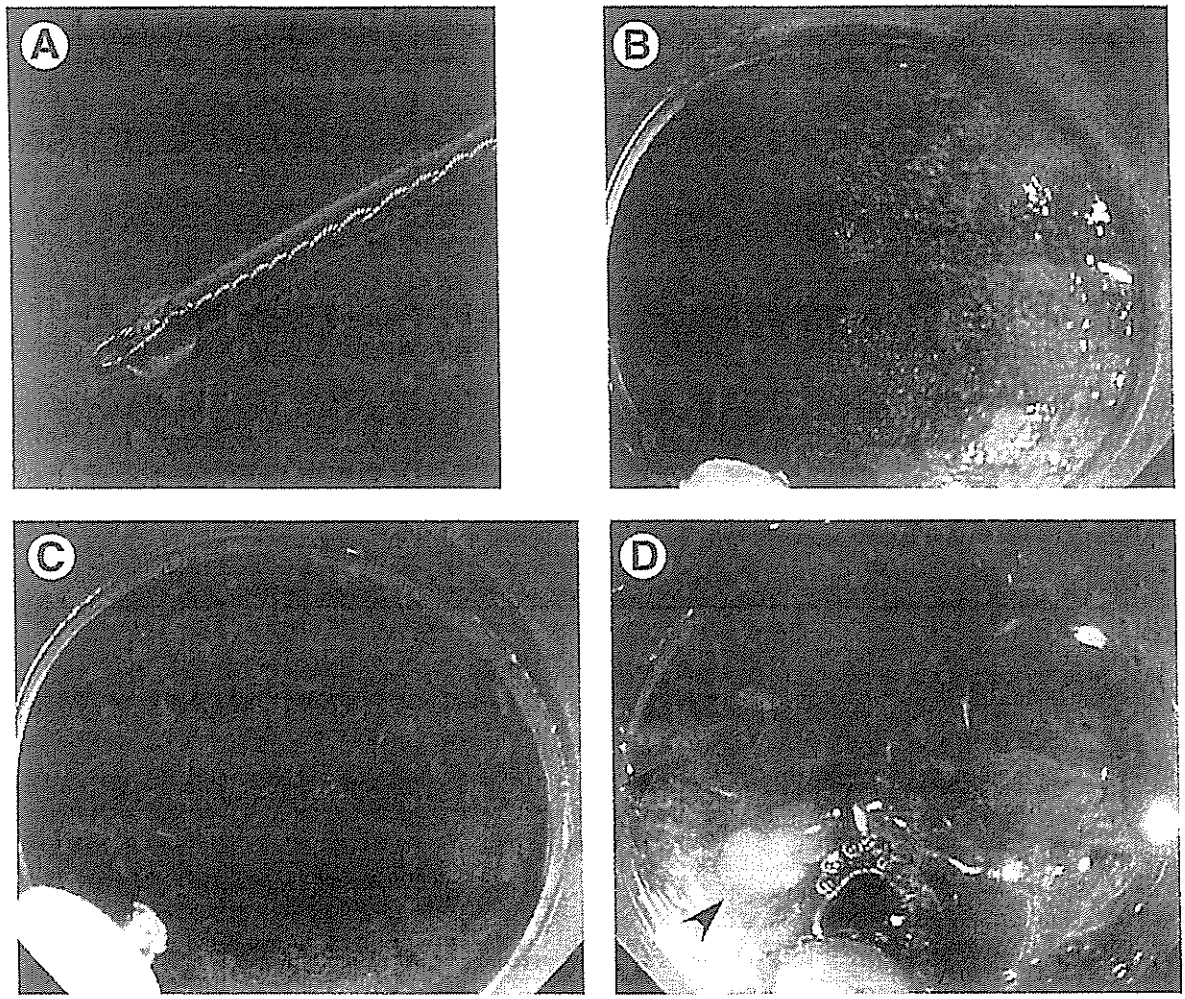

Figure 2. (*A*) Flexknife. (*B*) Tip of the Flexknife used for marking the circumference of the lesion. (*C*) Tip of the Flexknife used for the mucosal incision and submucosal dissection. (*D*) The proper esophageal glands observed in the submucosal layer (*arrowheads*).

the lesion, which could be visualized as the border between the stained and unstained areas. With the tip of an electrosurgical snare (thin type, SD-7p-1; Olympus, used from January 2002–December 2002) or Flex knife (KD-630L; Olympus, used from January 2003–September 2005) (Figure 2*A*),[14,15] dots placed about 5 mm outside of the lesion margin at 2-mm intervals were made to mark the circumference of the target lesion. The knife was fixed at a length of 1 mm (Figure 2*B*) and placed on the mucosal surface with little tension so as not to induce contact bleeding from the mucosa. Soft coagulation mode (output, 50 W) was used as the electronic current.

Creating a submucosal fluid cushion. One percent 1900 kd hyaluronic acid preparation (Suvenyl; Chugai Pharmaceutical Co, Tokyo, Japan) was mixed in normal saline (in use from January 2002–October 2003) or 10% glycerin plus 5% fructose and 0.9% saline preparation (Glyceol; Chugai Pharmaceutical Co) (in use from November 2003–September 2005). The solvent solution used for the hyaluronic acid preparation was changed because of novel knowledge of submucosal injection solutions.[18,19] Glyceol contains sugar that interacts with hyaluronic acid to increase the viscoelasticity of the hyaluronic acid solution over that of normal saline. The ratio of Glyceol and Suvenyl was also changed from a 1:3 ratio (used from January 2002–March 2004) to a 1:7 ratio (used from April 2004–September 2005) as a result of technical advances. To clarify the area of the submucosal injection, to distinguish clearly between the muscle layer and the submucosal layer, and to produce higher hemostatic ability, indigo carmine and epinephrine were added to the solution at concentrations of 0.005% and 0.0005%, respectively. About 2 mL of the solution was injected into the submucosal layer at a time, and the injection was repeated a few times until the mucosa that was intended to be incised was lifted to an acceptable level.

Incising the mucosa outside the lesion. After lifting the lesion, a mucosal incision was made with the tip of an electrosurgical snare (thin type) or Flexknife. The knife was fixed at a length of 2 mm (Figure 2C) and gently pressed onto the mucosa to produce a cutting effect by using the ENDOCUT mode with effect 2 (output, 60 W). The distal half of the mucosal incision was completed first, followed by the proximal half.

Dissecting the submucosal layer beneath the lesion. Before incising the entire circumference of the lesion, dissection of the submucosa was begun from the area in which the mucosal incision was completed to keep from flattening the lifted area as the procedure progressed. When a proper esophageal gland was observed during dissection, it was important to dissect at a level deeper than the gland to prevent resection with tumor-exposed basal margins (R1 [basal] resection) (Figure 2*D*). The principal knife used for the submucosal dissection was the same length as that used for the mucosal incision with forced coagulation mode (output, 40 W), and in difficult dissections the Hookknife (KD-620LR; Olympus)[20] was also used in combination with the principal knife. To control bleeding, hemostatic forceps (SDB2422; Pentax or Coagraspers FD-410LR; Olympus) were used in soft coagulation mode (output, 50 W). These hemostatic devices have a narrow opening angle, a small cup, and a blunt edge that look similar to small-sized hot-biopsy forceps. After pinpoint holding and mechanical compression, electrocoagulation is easily performed to obtain hemostasis. The water-jet system supplies a continuous jet of water at high pressure, which easily and swiftly washes away any blood obstructing the visual field, allowing identification of the vessel that is bleeding.

Treatment of artificial ulcer after endoscopic submucosal dissection. After resection of the lesion, visible vessels of the resulting artificial ulcer were treated with the hemostatic devices in soft coagulation mode (output, 50 W) to prevent delayed bleeding. Finally, sucralfate was sprayed onto the base of the ulcer by using the outer sheath of a clipping device that was inserted into the instrument channel of an endoscope both to confirm the hemostasis and to coat the surface of the ulcer.[21]

Three hours after ESD, patients were permitted to drink a small amount of water. The next day, if the patient's symptoms, laboratory findings, and chest x-ray were unremarkable, a light meal was permitted, and the patients were discharged within 1 week. If complications occurred, the schedules were changed according to the individual patient's condition.

All evaluations of esophageal SCNs were performed according to the Paris classification and revised Vienna classification.[22–24] To aid in the orientation, thin, curled-up ESD specimens were flattened and fixed at their periphery with thin needles pinning them to an underlying cork board before the specimens were immersed overnight in 10% formalin. The fixed specimens were then sectioned serially at 2-mm intervals parallel to a line that included the closest resection margin of the specimen, so that the resected margins and invasion depth could be assessed accurately. The sectioned materials were embedded in paraffin to make histologic sections that were stained with hematoxylin-eosin and were examined microscopically. The patients who had a histopathologic diagnosis of invasive carcinoma deeper than the lamina propria mucosae (the muscularis mucosae [m3], or the submucosa [sm] and/or vessel infiltration or incomplete resection on the basal margins, resection with nonevaluable tumor-free basal margins [Rx (basal) resection], or resection with tumor-exposed basal margins [R1 (basal) resection]) were recommended for additional treatments such as esophagectomy with lymph node dissection or radiation therapy with or without chemotherapy for possible lymph node metastases. Even if the histopathologic evaluation revealed that the lesions fulfilled the criteria of node-negative tumors but were incompletely resected on the lateral margins, resection with nonevaluable tumor-free lateral margins (Rx [lateral] resection), or resection with tumor-exposed lateral margins (R1 [lateral] resection), the patients were followed without additional treatments because the burn effects on the resected tissue or artifactual problems with processing the resected tissue sometimes made a precise histopathologic evaluation of the lateral margins difficult.

Follow-up endoscopy with iodine staining was usually performed about 2 months after ESD to confirm healing of the postprocedure ulcers and to exclude the presence of residual tumors, then again at about 6 months and 12 months after ESD, followed by annual endoscopic examinations to check for local recurrence and/or a second primary lesion for cases fulfilling the criteria of node-negative tumors. The existence of distant or lymph node metastases was evaluated with computed tomography and endoscopic ultrasonography indefinitely.

Results

The clinicopathologic features of the included patients are shown in Table 1. All the lesions were resected in an en bloc fashion. En bloc resection with tumor-free lateral/basal margins (R0 resection) was ac-

Table 1. Clinicopathologic Features of the Esophageal SCNs

		No. of SCNs
Mean size, *mm* (range)	24 (2–66)	
Location	Ce/Ut/Mt/Lt/Ae	0/7/29/17/5
Circumference of the esophageal lumen	<1/2/1/2 to <3/4/>3/4	39/12/7
Macroscopic type	IIa/IIb/IIc/IIc + IIa	2/20/35/1
Histologic depth	Dysplasia/m1/m2/m3/sm1/sm2	18/24/8/11/4/3
Vessel infiltration	Presence/absence	7/51

NOTE. The terms of macroscopic type and histologic depth are derived from reference 22. Ce, cervical esophagus; Ut, upper thoracic esophagus; Mt, middle thoracic esophagus; Lt, lower thoracic esophagus; Ae, abdominal esophagus; IIa, superficial, elevated type; IIb, flat type; IIc, superficial shallow, depressed type; m1, intraepithelial carcinoma; m2, microinvasive carcinoma (invasion through the basement membrane); m3, intramucosal carcinoma (invasion to the muscularis mucosae); sm1, superficial invasion (less than 200 μm below the muscularis mucosae) in the submucosa; sm2, middle invasion (more than 200 μm below the muscularis mucosae) in the submucosa.

complished in 45 of the 58 dissected lesions (78%) (Table 2). The mean resection size was 38 mm (range, 11–72 mm), and the mean lesion size was 24 mm (range, 2–66 mm). The small lesions for which conventional EMR seemed to be applicable in terms of lesion size were treated by ESD because of the single piece resection with coexisting lesions located nearby, or the existence of scarring caused by previous chemotherapy, radiation therapy, or endoscopic treatments. Fifty-three lesions (91%) were located in the thoracic esophagus, and 39 lesions (67%) were spread over less than half of the circumference of the esophageal lumen. Forty lesions (69%) (8 dysplasias, 24 m1, and 8 m2) were considered node-negative tumors by histopathologic evaluations of the ESD specimens.

Minor bleeding was encountered in all the dissections when incising the mucosa or dissecting in the submucosal layer, but hemostasis was achieved with thermocoagulation and without the use of clips during the procedure. A mean change in hemoglobin levels between pre-ESD and post-ESD of −0.22 g/dL (range, −1.5 to +1.3 g/dL) was observed, and the hemoglobin levels dropped by more than 1 g/dL in 7 (16%) of the 43 patients. No patient experienced massive hemorrhage requiring a blood transfusion or a postprocedure emergency endoscopy.

Perforation, which was diagnosed by endoscopic findings of tearing of the proper muscle layer, occurred in the dissection of 4 (6.9%) lesions, and all cases of perforation were accompanied by pneumomediastinum. The size of all 4 perforations was less than 5 mm, which was measured by comparison with the tip of an electrosurgical knife. After immediate closure of the perforations by endoscopic clipping and completion of ESD, conservative treatments with intravenous antibiotics and allowing no oral intake were prescribed by the primary physician who advised the patients and their family after informed consent. Leukocytosis returned to within a normal range after a few days without any evidence of mediastinitis in all the patients with perforation. Re-feeding was begun gradually with pureed foods, and antibiotics were subsequently stopped. Endoscopy or fluorography was not performed to confirm the complete sealing of the perforation before beginning oral feeding because all the perforations were treated with immediate closures with clips. Pneumomediastinum disappeared spontaneously within a week in all the patients, which was confirmed by chest x-ray. All perforations occurred before July 2003, before the techniques and experience with esophageal ESD were completely established. No patients experienced pneumomediastinum without perforation, which was confirmed by chest x-rays taken after ESD.

Nine lesions in 9 (16%) patients required several (median, 3; range, 1–16) sessions of periodic balloon dilatation after ESD for esophageal stricture, which was repeated every 1–2 weeks after dysphagia was recognized. ESD for 7 lesions that spread over more than three fourths of the circumference of the esophageal lumen, which required semicircular or complete circular resection, caused esophageal stricture requiring balloon dilatation. All the postprocedure strictures were successfully managed endoscopically.

Of 40 lesions in 31 patients that fulfilled the criteria of node-negative tumors, 22 lesions in 18 patients were successfully followed in our hospital for more than 6 months by endoscopy. The reasons for excluding the other lesions from the follow-up analysis were due to 4 with concurrent lesions that required additional treatments, 8 were followed up at another hospital, and 6 had

Table 2. En Bloc Resection Rate and Histologic Margin of the Resected Specimens

	Lesions	
	n	%
En bloc resection	58	100
R0 resection	45	78
R1 (lateral) resection (tumor extending to lateral margins)	5	8.6
R1 (basal) resection (tumor extending to basal margins)	2	3.4
Rx (lateral) resection (not evaluable for lateral margins)	6	10
Rx (basal) resection (not evaluable for basal margins)	0	0

a follow-up duration of less than 6 months. During a mean follow-up duration of 17 months (range, 6–36 months), only 1 noninvasive carcinoma with Rx (lateral) resection (4.5%) recurred locally 6 months after ESD as noninvasive carcinoma. This lesion was completely resected by a second ESD, and an additional follow-up of 12 months' duration revealed no further local recurrence. No lymph node or distant metastases were observed.

Of 18 lesions in 16 patients with concomitant risks of nodal metastases, 6 lesions in 5 patients were closely followed up without additional treatment because of the patient's decision. All of these lesions were intramucosal invasive carcinomas into the muscularis mucosae (m3), and only 1 lesion had lymphatic vessel infiltration, which increased the possibility of nodal metastasis. During a mean follow-up of 15 months' duration (range, 6–23 months), the lesion with lymphatic vessel infiltration recurred in the regional lymph nodes 18 months after ESD as a nonresectable, recurrent tumor. The patient was followed with computed tomography every 6 months and with annual endoscopic ultrasonography. The lymph node swelling was not detected by endoscopic ultrasonography and computed tomography 6 months before its detection.

Discussion

To show the efficacy of the ESD procedure for esophageal SCNs, 2 aspects, the technical feasibility of the procedure and follow-up data showing the efficacy of the procedure, have to be considered. Although the duration of follow-up is short, the present study shows that no patient with esophageal SCNs that met the criteria of node-negative tumors postoperatively treated with ESD experienced recurrence extraluminally. One noninvasive carcinoma with Rx (lateral) resection recurred locally in the epithelial layer, but it was successfully treated by a second ESD procedure. Our data suggest that ESD can be a successful treatment for esophageal SCNs fulfilling the criteria of node-negative tumors. Furthermore, considering the lack of complications, ESD could be a relatively safe procedure for most patients. The perioperative mortality rate was zero, and the most serious postoperative complication was benign stricture of the esophagus, which was successfully treated with balloon dilatation. Although 4 patients had small perforations, these were managed successfully without surgical rescue. We emphasize again that all the perforations occurred during the early period of this study before the procedure was perfected, and no further perforations have occurred for more than 2 years. The esophagus is one of the most accessible sites for any instrumentation and is fixed in the retromediastinum. Hence, the endoscopic approach is easier, and the technical difficulty is less than that for other gastrointestinal organs such as the stomach and colon. When we master the strategy for the esophagus, ESD might be safely performed in this location because the other serious complication, namely bleeding, is also considerably less than that observed in the stomach.

The major drawbacks of conventional EMR such as strip biopsy[25] and EMR with cap[26] are local recurrences, which are reported in up to 20% of EMR series.[27] The reason why esophageal SCNs treated by conventional EMR recur locally at such a considerable frequency is unknown, but we speculate that one of the reasons is multifragmental resection by EMR, which might leave tumor cells between the spaces of resected mucosa and which is impossible to evaluate through the histology of the resected specimens. Another possibility might be the incomplete resection of ductal extensions of tumor cells into the proper esophageal glands. It is known from thorough histologic analysis that ductal extension of tumor cells in the proper esophageal glands has no risk of nodal metastases, which is considered to be noninvasive carcinoma even if the tumor cells are observed in the submucosal layer.[28] Both situations are preventable by applying ESD for local treatment rather than conventional EMR, because the en bloc resection allows for a check of the tumor margin, even when the lesion has a complex shape or a large size, and ductal extension of tumor cells into the glands can removed by resecting the submucosal connective tissues beneath the proper esophageal glands with direct endoscopic views.

In summary, this study shows that ESD is a promising technique that is not limited to the stomach, but it can also be used for the resection of esophageal SCNs after refinement of the technique. However, further evaluation and assessments of case series are necessary before ESD can be widely accepted as a standard endoscopic treatment for esophageal SCNs.

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INTERNAL MEDICINE

□ CASE REPORT □

Nonparasitic Solitary Giant Hepatic Cyst Causing Obstructive Jaundice was Successfully Treated with Monoethanolamine Oleate

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Abstract

A 77-year-old man hospitalized for epigastric pain showed jaundice of the skin and conjunctivae. Laboratory tests revealed elevated hepatobiliary enzymes and inflammatory markers, and imaging studies demonstrated a 12 cm hepatic cyst compressing the common bile duct. The diagnosis was a giant hepatic cyst causing obstructive jaundice. Cyst drainage and sclerotherapy with 5% monoethanolamine oleate was performed twice, resulting in almost complete disappearance of the cyst. Obstructive jaundice due to a hepatic cyst, as seen in this case, is relatively rare and this report includes a review of other similar cases in Japan.

Key words: hepatic cyst, obstructive jaundice, monoethanolamine oleate

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Introduction

Hepatic cysts are usually asymptomatic but may occasionally present as abdominal pain, nausea, vomiting, and abdominal distention (1, 2). However, even in symptomatic hepatic cysts, obstructive jaundice is rarely seen. Sanfelippo et al (3) reported obstructive jaundice in only two of 82 patients with hepatic cysts. Recent trends in the treatment of symptomatic hepatic cysts, except in cases of acute rupture, hemorrhage or where cancer is suspected, include cyst drainage followed by drug injection (sclerotherapy). Ethanol and minocycline are often used as sclerosing solutions, and more recently, monoethanolamine oleate has been used with good results (4-6). We recently encountered a patient with obstructive jaundice due to a hepatic cyst who was successfully treated with sclerotherapy using monoethanolamine oleate. This case is presented here, together with a discussion of the related medical literature.

Case Presentation

A 77-year-old man was referred to our hospital because of persistent epigastric pain. His past history was unremarkable except for appendectomy at age 18 and pulmonary tuberculosis at age 75. Physical examination on admission showed the patient to be lucid and afebrile. His blood pressure was 152/84 mmHg and pulse rate 87 bpm. The abdomen was soft and slightly distended, with mild tenderness in the upper abdomen. There was no rebound, guarding, hepatosplenomegaly, palpable masses, or lower extremity edema. Laboratory findings on admission revealed elevated hepatobiliary enzymes and inflammatory markers (Table 1), urinalysis was positive for bilirubin, and abdominal ultrasound showed a large cystic lesion in the right hepatic lobe (Fig. 1). The lesion contained no septum or calcifications, and the intrahepatic bile ducts in both hepatic lobes were dilated. Abdominal computed tomography confirmed the presence of a large cystic lesion 12 cm in diameter (Fig. 2a),

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Table 1. Laboratory Data on Admission

Urine		*Blood chemistry*			
Protein	(-)	AST	430 IU/l	Na	142 mEq/l
Sugar	(-)	ALT	492 IU/l	K	3.5 mEq/l
Occult blood	(-)	γ-GTP	692 IU/l	Cl	106 mEq/l
Bilirubin	(3+)	LDH	340 IU/l	BUN	26 mg/dl
Peripheral blood		ALP	1884 IU/l	Cr	0.9 mg/dl
WBC	10600 /mm³	T.Bil	7.0 mg/dl	T.Chol	214 mg/dl
Neutro 75.6%		D.Bil	6.0 mg/dl	TG	49 mg/dl
Lym 17.1% Mo 6.2%		AMY	72 IU/l	PT/INR	1.01
Eo 0.9% Ba 0.2%		TP	6.5 g/dl	APTT	26.1 sec.
RBC	468x10⁴ /mm³	Alb	4.1 g/dl	CEA	2.2 ng/ml
Hb	14.7 g/dl	Ch-E	274 IU/l	CA19-9	10 U/ml
Ht	44 %	CRP	2.8 mg/dl	HBs Ag	(-)
Plt	25.2x10⁴ /mm³	ESR	25 mm/hr	HCV Ab	(-)

which showed no enhancement with contrast medium (Fig. 2b). Both T1- and T2-weighted magnetic resonance images of the lesion showed homogeneously high signal intensity as compared with normal liver parenchyma (Fig. 3a and b). Magnetic resonance cholangiopancreatography indicated the presence of a large spherical lesion near the confluence of the right and left hepatic ducts that was compressing the intrahepatic bile ducts and keeping them separate from the common bile duct (Fig. 3c). Endoscopic retrograde pancreatocholangiography revealed downward compression of the common bile duct, gall bladder, and cystic duct (Fig. 4). The intrahepatic bile ducts could not be visualized on endoscopic retrograde pancreatocholangiography. No flow of contrast medium was detected in the cystic lesion, and neither gall stones, tumors, nor abnormalities of the pancreatobiliary duct could be identified. On the basis of these findings, a diagnosis was made of obstructive jaundice due to a giant hepatic cyst.

On day 15 after admission, about 600 mL of fluid was drained from the cyst by means of transcutaneous transhepatic drainage. Repeat abdominal ultrasound showed almost complete disappearance of the cyst (Fig. 5). Sixty milliliters of 5% monoethanolamine oleate was then injected, the patient was placed in different positions for 30 minutes, after which the monoethanolamine oleate was aspirated. On the following day the drainage tube was removed. The fluid drained from the cyst was reddish-brown and serous, with cell counts of less than 100 (cells could not be classified), a specific gravity of 1.019, negative Rivalta test result, and cytologically identified as class I. Bacteriologic cultures of the cyst contents were negative, but cyst fluid tumor markers were markedly elevated: CEA, 94.7 ng/mL; CA19-9, ≥ 5,000 U/mL; CA125, 1,159 U/mL. Despite the initial almost complete disappearance of the cyst, regrowth was noted 28 days after the first drainage (Fig. 6), and a second drainage was thus performed. Drainage of about 300 mL of fluid followed by injection of 60 mL of monoethanolamine oleate resulted in disappearance of the hepatic cyst and resolution of the abdominal pain. On days 33 and 41 after admission, serum transaminase and total bilirubin, respectively, were within normal limits. On day 41 after admission, the patient was discharged from hospital. He has since shown an uneventful course with no recurrence of the hepatic cyst.

Discussion

The prevalence of hepatic cysts is 0.1 to 0.5% (3) based on autopsy studies and 2.5% based on ultrasound examinations (7). Hepatic cysts have been classified by Henson et al (8) into four types: congenital, neoplastic, inflammatory, and traumatic. Congenital hepatic cysts are further categorized into solitary and polycystic cysts. The findings of the present patient are consistent with a solitary unilocular cyst.

For diagnosing a hepatic cyst, it is important to rule out hepatic cystadenoma or cystadenocarcinoma. Imaging study findings that suggest a cystadenoma or cystadenocarcinoma include the presence of solid elements with enhancement on contrast computed tomography (9). In the present patient, none of the imaging studies showed solid elements, and no enhancement was seen on contrast computed tomography. The diagnosis in this case was therefore a simple hepatic cyst. Laboratory findings on admission showed elevated hepatobiliary enzymes and inflammatory markers. In addition, serum transaminase was elevated, but normalized after cyst drainage. Since other causes of an elevated serum transaminase level were absent, this elevation was considered to be caused by obstructive jaundice.

Typical magnetic resonance imaging findings of a hepatic cyst include the same signal intensity as water (10), that is, homogeneous low signal intensity on T1-weighted imaging and homogeneous high signal intensity on T2-weighted imaging. However, our patient had homogeneous high signal intensity on both T1- and T2-weighted images, which suggests intracystic hemorrhage or a high protein concentration. This, combined with the reddish-brown color of the drained cyst fluid, indicates that the patient may have been suffering intracystic bleeding, which caused cyst enlargement prior to the development of symptoms, even though findings of abdominal ultrasound and abdominal computed tomography were not typical for intracystic hemorrhage.

Markedly elevated tumor marker levels have been reported even in histologically diagnosed simple cysts (11, 12). Iwase et al (13) reported CA19-9 levels in hepatic cyst fluid at least 100 times higher than in normal serum concentrations. The present patient also had markedly elevated cyst fluid tumor marker levels, and imaging studies indicated a simple cyst.

Solitary nonparasitic cysts of the liver causing obstructive jaundice were first reported in 1950 by Caravati et al (14). In connection with the findings for our patient, we reviewed reports of similar cases in the Japanese and English medical literature. We found a total of 51 patients with the following characteristics: mean age, 65.8±15.2 years (range, 29 to 90 years); male to female ratio, 7:6; cyst size, 12.5±5.1 cm (range, 2 to 30 cm); and total bilirubin, 10.2±7.7 mg/dL (range, 1.5 to 31.5 mg/dL). Twenty-nine patients were treated surgically and 22 were treated non-surgically. Of the