総特集

予防医学はどこまで可能か

がん検診受診率と課題

京都府立医科大学大学院
医学研究科地域保健医療疫学
渡邊能行

要旨：わが国のがん検診は対象者の20～25％が各部位（胃、肺、大腸、子宮、乳房）のがん検診を毎年受診している。地域における大腸がん検診の精検受診率は約60％と低く、問題である。今後、より正確な受診率の把握と未受診者対策を進めていく必要がある。

がん検診は83年に胃と子宮が老人保健法の中で保健事業として位置づけられたのを皮切りに、87年には肺と乳房、さらに92年には大腸と拡大されてきた。しかし、98年には財政上の理由から補助金が廃止され、市町村は厳しい財政の中で何とか継続しているというのが現状である。他方、21世紀の国民健康づくり運動である「健康日本21」においては、生活習慣病対策として1次予防の強化が謳われている一方で、2次予防としてのがん検診については受診者の5割以上の増加が目標値として示されている。

本稿では、受診率をキーワードとしてがん検診の現状について触れてみたい。

各部位のがん検診受診率は20～25％

地域保健・老人保健事業報告[1]による00年～02年度、胃、肺、大腸、子宮、乳房のがん検診受診率について図1に示す。これは、全国の市町村が保健事業として行っているがん検診を厚生労働省がまとめたものである。各年のがん検診受診率は、胃で約13％、肺で約23％、大腸で約16％、子宮で約14％、乳房で約12％となっていた。大腸がん検診の受診率は短い上記3年間で増加傾向を示していたのが若干目立つ程度で、他の4部位のがん検診は各年ともほぼ同程度の受診率である。

この受診率については実は分母が難しい。すなわち、市町村が対象としているのは胃、肺、大腸は40歳以上の男女であり、子宮と乳房は30歳以上の女性であるが、基本的に職域においてがん検診を受診する機会のある者は対象外であり、国民健康保険加入者とその家族及び社会保険加入者の家族が対象となっている。

しかし、市町村の保健担当課が対象者をきちんと把握しているかといえば、それはかなりのばらつきがある。人口規模の小さい町村においては、きちんとした把握はそれなりの努力をすれば可能であるが、人口の大きい都市においては困難な作業を伴う。都道府県によっては、国保加入率や性別・年齢階級別人口を基に計算式を用いて対象人口を推計している市町村があるようである。そういう意味で、地域保健・老人保健事業報告[1]によるがん検診受診率もなかなか解釈に難しい統計値という印象がある。

他方、最近国民に過去1年間のがん検診受診の有無を回答してもらうという調査に基づく受診者数も示されるようになった。それが、国民生活基礎調査[2]のがん検診受診者数である。いわゆる3年に1回の大規模調査であった01年度の「健康票」の中で、「あなたは過去1年間にがん検診を受けましたか」という設問があり、胃がん検診、肺がん検診、大腸がん検診、子宮がん検診、乳がん検診の

●Summary
Recent annual participation rate in cancer screening programs (stomach, lung, colon and rectum, uterus, and breast) in Japan was about 20－25%. Participation rate in further examination for colorectal cancer was low and about 60%. It is necessary to reveal accurate participation rate in cancer screening programs and to screen people who have never participated in the programs.

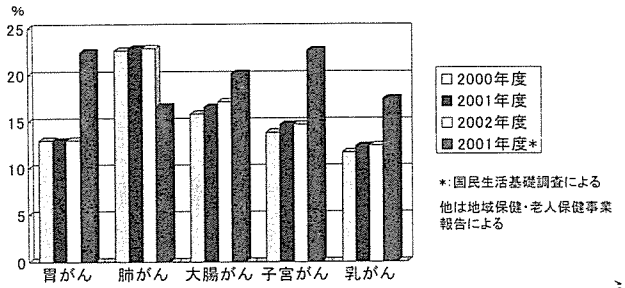

図1　部位別がん検診の受診率

表1　がん検診の受診率（1997年）

がん検診の種類	胃がん	肺がん	大腸がん	子宮がん	乳がん
地域集団における受診率*[1]	13.8%	22.4%	14.6%	15.2%	12.7%
地域集団における受診者数*	4,272,814	7,061,535	4,872,954	3,766,047	3,228,771
職域集団における推定受診率**	31.2%	9.8%	16.6%	32.0%	27.6%
職域集団における推定受診者数**	11,821,429	3,729,600	6,281,173	6,343,703	5,488,505
地域・職域集団の推定受診者数	16,093,814	10,791,535	11,153,954	10,109,750	8,717,276
地域・職域集団の推定受診者の受診率	25.3%	17.0%	17.5%	24.3%	20.9%

*：厚生省大臣官房統計情報部「平成9年度老人保健事業報告」[3]
**：労働省大臣官房政策調査部「平成9年労働者健康状況調査報告」[4]と同年の性・年齢階級別就業者数から40歳以上について（子宮がんと乳がんは30歳以上について）推定

5つの回答選択肢から複数回答を可として○をつけてもらう調査が実施された。胃、肺、大腸は40歳以上の男女について、子宮と乳房は30歳以上の女性についての受診者数を01年の当該年齢人口で除し、同じ表1に示す。

受診率は、胃がん検診で約22%、肺がん検診で約17%、大腸がん検診で約20%、子宮がん検診で約23%、乳がん検診で約17%となっていた。肺がん検診を除く他の4つのがん検診では国民生活基礎調査[2]による受診率の方が地域保健・老人保健事業報告[1]による受診率よりも高値であり、それは職域での受診者も受診したと回答しているためと解釈される。

地域保健・老人保健事業報告[1]による受診率よりも低値となった肺がん検診については、胸部X線検査を受けていても肺がん検診と認識していない国民が多く存在するためであろうと思われる。今後、この設問はがん検診の受診の有無を質問するのではなく、がん検診のスクリーニング検査の受診の有無を質問するように改変されるべきであろう。

地域と職域を併せたがん検診の受診率を明らかにするために、97年度の老人保健事業報告[3]と97年の労働者健康状況調査報告[4]を用いて推計した結果[5]を表1に示す。胃がんが25・3%と最も高く、続いて子宮がん24・3%、乳がん20・9%、大腸がん17・5%、肺がん17・0%の順であった。年が異なるが01年の国民生活基礎調査[2]と似かよった大きさの受診率であった。国民のおおよそ20～25%が各部位のがん検診を毎年受診しているというのが現状である。

精密検査受診率の低さは大きな問題

00年度の地域保健・老人保健事業報告[1]資料において、要精検者のうち結果別人員の報告が「異常認めず」「がんであった者」「がんの疑いのある者」「がん以外の疾患であった者」の合計の人数の割合である精検受診率は、胃がん76・5%、肺がん77・5%、大腸がん59・2%、子宮がん67・7%、乳がん78・6%であった[1,5]。

大腸がん検診の精検受診率が低いのは、大腸内視鏡検査や注腸X線検査といった精密検査の方法が受診者にとって負担の重いものであることと、その検査を提供できる医療機関が地域によっては限定され、処理能が十分でないこと、逐年検診受診者で毎年便潜血陽性の者が負担の重い精密検査の受診を忌避していること等が影響していると考えられるが、40%以上もの要精検者が精密検査を受診していないことは、検診のやりっぱなしであり、大きな問題である。

02年度の日本対がん協会の資料[6]によれば、大腸がん検診の精検受診率は地域の住民検診で71・8%、職域検診で41・9%と、職域の方が地域よりもさらに低値となっていた。大腸がん検診の精検受診率については、真剣に対策を講ずる必要があると考える。

受診率向上は未受診者対策から

国民のおおよそ20～25%が各部位のがん検診を毎年受診しているという現状に対して、「健康日本21」では受診者の5割増加を目指している。受診者が増加することは、精密検査の受診が伴えば2次予防が期待できるので、基本的には良いことであるが、どのようにすれば実現可能であろうか。

その前に、そもそも欧米での現状はどうなっているのだろうか。米国のCancer Control Planetのホームページには、例えば、00年のデータとして乳がんのスクリーニングであるマンモグラフィーを過去2年以内に受けたことのある50歳以上の女性の割合が州別に示されている。その数値は最低のワイオミングで70・9%であり、最高のデラウエアではなんと90・3%であった。

ここには参考となることが2点ある。この統計は全米各州における標本調査であり、人口規模にもよるが数百から数千の個人を対象に、スクリーニングそのものの受診の有無ではなくスクリーニング検査であるマンモグラフィーの受診の有無を質問していることと、過去1年間ではなく過去2年間について尋ね

ていることである。

具体的なスクリーニング検査の受診の有無を尋ねることで回答がより明確になり、国民生活基礎調査の中で肺がん検診受診者が過小評価されていることへの対応ともなり得、このような工夫を通してより正確な受診率の推定が必要であると考える。臨床におけるスクリーニング検査受診有の者を加算すれば、わが国のがん検診に用いられるスクリーニング検査の受診率はもっと高いのかもしれない。

また、わが国よりも罹患率の高い米国の乳がんにおいてすら、スクリーニングは過去2年以内に1回以上を受診ありとしていることである。これは12～33カ月ごとのマンモグラフィーによるスクリーニングが乳がんの死亡率を減少させていたというHumphrey LLらによる根拠[7]に基づいた判断である。わが国におけるがん検診は基本的に逐年検診として設計されているが、適正な受診間隔の検討も今後行っていく必要がある。

地域保健の現場では、胃がんや大腸がんによって不幸な転帰をとられた方の胃がん検診や大腸がん検診の受診歴を調べてみると、全く受診したことがなかったという例によく遭遇する。また、糖尿病で医療機関を長期受診している患者さんから、症状発見の進行胃がんが診断され、カルテをよく調べてみると、糖尿病についてはきちんと医療を受けていたのに、胃の検査を一度も受けたことがないといった事例も時に聞く。

そのようなことを考えると、やみくもに受診率向上を模索するのではなく、全くがん検診を受診したことのない未受診者のがん検診受診を勧めることを通した受診率向上を考えていく必要があるのではないかと考える。すなわち、がん検診を単独の保健サービスとして位置づけるのではなく、医療におけるスクリーニング検査の受診も含めて、トータルのがん対策として見直していくことも意味があるのではないかとも思う次第である。

参考文献

1 平成12～14年度地域保健・老人保健事業報告、厚生省大臣官房統計情報、厚生統計協会、2002～2004。
2 平成13年国民生活基礎調査、厚生省大臣官房統計情報、厚生統計協会、2003。
3 平成9年度老人保健報告、厚生省大臣官房統計情報、厚生統計協会、1999。
4 平成9年労働者健康状況調査報告、労働省大臣官房政策調査部、労働省大臣官房政策調査部第二課、1998。
5 渡邊能行、森田益次：わが国のがん検診の実施現状。住民検診・職域検診・人間ドックのためのがん検診計画ハンドブック（三木一正、渡邊能行編）。南江堂、2-7, 2004
6 平成14（2002）年度がん検診の実施状況、日本対がん協会、2003
7 Humphrey LL, et al: Screening for Breast Cancer. Systematic Evidence Review No. 15 (Prepared by the Oregon Health & Science University Evidence-based Practice Center under Contract No. 290-97-0018). Rockville, MD: Agency for Healthcare Research and Quality. September 2002. (Available on the AHRQ Web site at: www.ahrq.gov/clinic/serfles.htm).

※ ※

渡邊能行（わたなべ・よしゆき）◎53年京都府生まれ。78年京都府立医大卒。86年京都府園部保健所長、93年京都府立医大公衆衛生学助教授、98年同大附属脳・血管系老化老化研究センター社会医学・人文科学部門教授、03年同大学院地域保健医療疫学教授。

Clinicopathologic and genetic characteristics of gastric cancer in young male and female patients

SHOGO SASAO[1], TORU HIYAMA[4], SHINJI TANAKA[3], MASAHARU YOSHIHARA[4], WATARU YASUI[2] and KAZUAKI CHAYAMA[1]

[1]Department of Medicine and Molecular Science; Division of Frontier Medical Science, [2]Department of Molecular Pathology, Division of Molecular Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University; [3]Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551; [4]Health Service Center, Hiroshima University, 1-7-1 Kagamiyama, Higashihiroshima 739-8521, Japan

Received December 6, 2005; Accepted February 9, 2006

Abstract. The pathways of gastric cancer in young patients (40 years of age or younger) have not yet been determined. We therefore examined clinicopathologically and genetically 68 gastric cancers in young patients and 66 tumors in older patients (41 years of age or older). Mutations in B-*raf* and K-*ras* were identified by PCR-SSCP following sequencing. Microsatellite instability (MSI) and *hMLH3* mutations were also examined. Histopathologically, diffuse-type gastric cancer and cancer in the whole of the stomach were found significantly more often in young patients than in older patients (21% vs. 2%, P=0.0006, and 77% vs. 32%, P<0.0001, respectively). Genetically, MSI and *hMLH3* mutations were found significantly more often in tumors in young patients than in tumors in older patients (15% vs. 4%, P=0.040, and 9% vs. 0%, P=0.036, respectively). Tumors in young female patients were found significantly less often in the lower-third of the stomach and showed a significantly greater frequency of MSI, compared to tumors in young male patients (33% vs. 9%, P=0.046, 5% vs. 30%, P=0.010, respectively). These results suggest that the pathways of gastric carcinogenesis differ between young patients and older patients, and that the pathways differ between the sexes in young patients.

Introduction

Gastric cancer has been decreasing in incidence over the last decade, but it is still the second most common cause of cancer-related death worldwide (1). It occurs most frequently in individuals 50-70 years of age. Gastric cancer results from a combination of environmental factors and accumulation of specific genetic alterations. Environmental factors, such as *Helicobacter pylori (H. pylori)* infection and a high-salt diet, and genetic factors play important roles in gastric carcinogenesis (2,3). Genetic alterations, such as activation of oncogenes K-*ras* and B-*raf* and inactivation of tumor suppressor gene *p53*, play important roles in the development of gastric cancers (4). Dysfunction of DNA mismatch repair genes, which leads to microsatellite instability (MSI), also plays a crucial role (5).

Gastric cancers occurring in young patients (40 years of age or younger) account for less than 5% of all gastric cancers (6,7). There have been several reports comparing clinicopathologic and biologic characteristics of young patients and older patients (more than 40 years of age) (8). Young patients with gastric cancer, in comparison to older patients, are thought to show a more aggressive clinical course and have a poorer prognosis. Thus, gastric cancers in young patients may have different genetic profiles from those in older patients. Although genetic characterization of gastric cancers has been the focus of several studies, few have addressed this issue specifically in young patients. The genetic pathways in young patients have not yet been determined. We therefore examined genetic alterations in gastric cancers to clarify differences in the disease between young patients and older patients.

Materials and methods

Study subjects were 134 patients with gastric cancer (68 young patients and 66 older patients) treated surgically at Hiroshima University Hospital or an affiliated hospital during the period 1990 through 2004. The young patients with gastric cancer were enrolled consecutively, and the older patients were enrolled randomly. For each patient, both cancerous and normal tissues were obtained at surgery.

Four-micrometer-thick sections were prepared from formalin-fixed, paraffin-embedded specimens. The sections were stained with hematoxylin and eosin (H&E) for histologic examination. Gastric cancers were classified as intestinal-

Correspondence to: Dr Shinji Tanaka, Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
E-mail: colon@hiroshima-u.ac.jp

Key words: gastric cancer, microsatellite instability, *hMLH3*, B-*raf*, K-*ras*

Table I. Primer sets used in the present study.

Gene	Sequence
K-*ras*	sense: 5'-TTGTTGGATCATATTCGTCC-3' antisense: 5'-TCAAAGAATGGTCCTGGACC-3'
B-*raf*	
exon 11	sense: 5'-AAACACTTGGTAGACGGGAC-3' antisense: 5'-ACTTGTCACAATGTCACCACATT-3'
exon 15	sense: 5'-CTTCATGAAGACCTCACAGT-3' antisense: 5'-GGCCAAAAATTTAATCAGTGGA-3'
hMLH3	
codon 583-585	sense: 5'-GCCTTTTGCAACAACATTATGG-3' antisense: 5'-GTGGAACATAATTTAACTCGCC-3'
codon 672-674	sense: 5'-AGACATCAAAGATTTAGCCAGC-3' antisense: 5'-CTGTAGGTTCATTCTCTAGCC-3'
BAT26	sense: 5'-TGACTACTTTTGACTTCAGCC-3' antisense: 5'-AACCATTCAACATTTTTAACCC-3'

type or diffuse-type as defined by Lauren (9). Depth of invasion was classified as to the mucosa or submucosa (early stage) or to the muscularis propria or deeper (advanced stage). The presence of lymph node metastasis was also examined. To analyze the relationship between tumor location and genetic alterations, the stomach was divided into three parts: the upper, middle, and lower parts. *H. pylori* infection was examined histologically with Giemsa staining. The presence of follicular gastritis, a type of *H. pylori*-associated gastritis characterized by the presence of prominent lymphoid follicles in the mucosal layer of the stomach (10), was also examined in the patients.

Ten-micrometer-thick tissue sections were placed on glass slides and stained with H&E. The tissue sections were then dehydrated in graded ethanol solutions and dried without a cover glass. Cancerous and normal tissues on the slides were scraped up with sterile needles, separately, by a microdissection technique. DNA was extracted from the tissues with 20 μl of extraction buffer [100 mM of Tris-HCl; 2 mM of ethylene diamine tetraacetic acid (EDTA), pH 8.0; 400 μl/ml of proteinase K] at 55°C overnight. The tubes were boiled for 7 min to inactivate the proteinase K, and then 2 μl of the extracts was used for each polymerase chain reaction (PCR) amplification.

Each tumor was evaluated for MSI by analysis of the mononucleotide repeat, BAT26. The microsatellite assay was performed as described elsewhere (11). The primer sets used in the present study are shown in Table I. Briefly, each 15 μl reaction mixture containing 10-20 ng of genomic DNA; 6.7 mM of Tris-HCl, (pH 8.8); 6.7 mM of EDTA; 6.7 mM of $MgCl_2$; 0.33 μM of primer labeled with (γ-^{32}P)dATP; 0.175 μM of unlabeled primer; 1.5 mM of each deoxynucleotide triphosphate; and 0.75 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ) was amplified for 40 cycles as follows: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and strand elongation at 72°C for 30 sec. The PCR products were electrophoresed on 6% polyacrylamide-8 M urea-32% formamide gels and autoradiographed overnight at -80°C with Fuji RX film. When additional bands appeared in the tumor DNA on the BAT26 marker, the tumor was defined as MSI-positive. Two mononucleotide repeats (poly A tracts) of *hMLH3* were also examined (12).

B-*raf* and K-*ras* genes were also examined. PCR-single-strand conformation polymorphism (SSCP) analysis was performed as described previously (4). The aberrant migration band on the SSCP gel was removed, amplified again, and directly sequenced on both strands with an ABI PRISM 310

Table II. Clinicopathologic characteristics of young and older patients with gastric cancer.

Characteristics	Young patients	Older patients	P-value
Sex			
Male/female	39/29	42/24	0.46
Tumor location			
Whole stomach/other/NA	11/42/15	1/65/0	0.0006
Histology			
Intestinal/diffuse	16/52	45/21	<0.0001
Tumor depth			
Early/advanced/NA	25/40/3	37/26/3	0.022
Helicobacter pylori infection			
Positive/negative	60/8	66/0	0.004
Follicular gastritis			
Present/absent	4/64	0/66	0.063

NA, information not available.

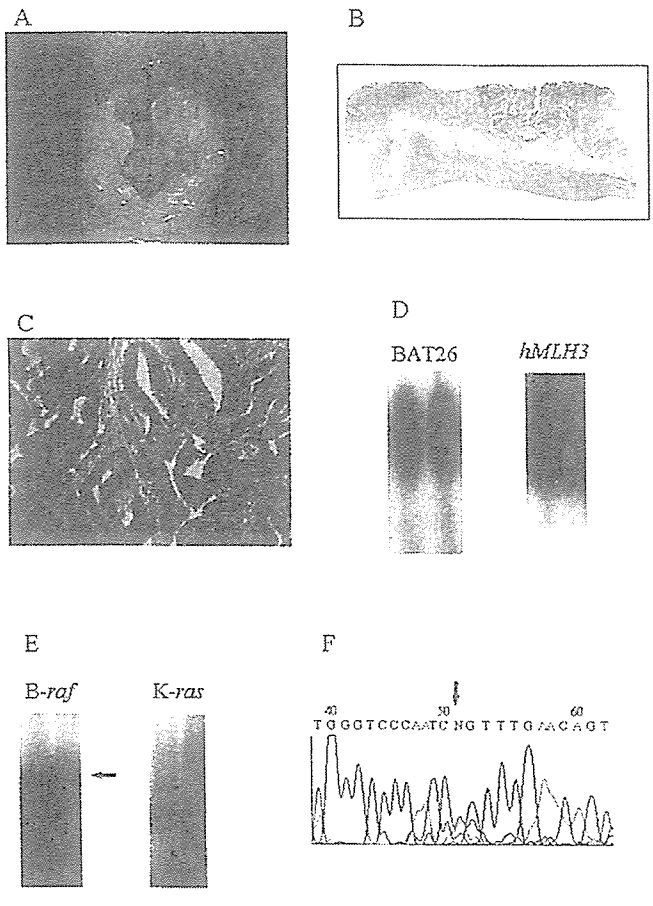

Figure 1. A representative case of intestinal-type gastric cancer. (A) Endoscopy revealed an ulcerated lesion at the greater curvature of the antral region. (B) Loupe appearance. (C) A histologically well-differentiated adenocarcinoma (x200) was identified. (D) Microsatellite analysis showed no alteration at BAT26 or *hMLH3*. (E) PCR-SSCP analysis showed mobility shift of B-*raf*. (F) Sequencing analysis of B-*raf* showed a CAG to CCG mutation at codon 608 of exon 15.

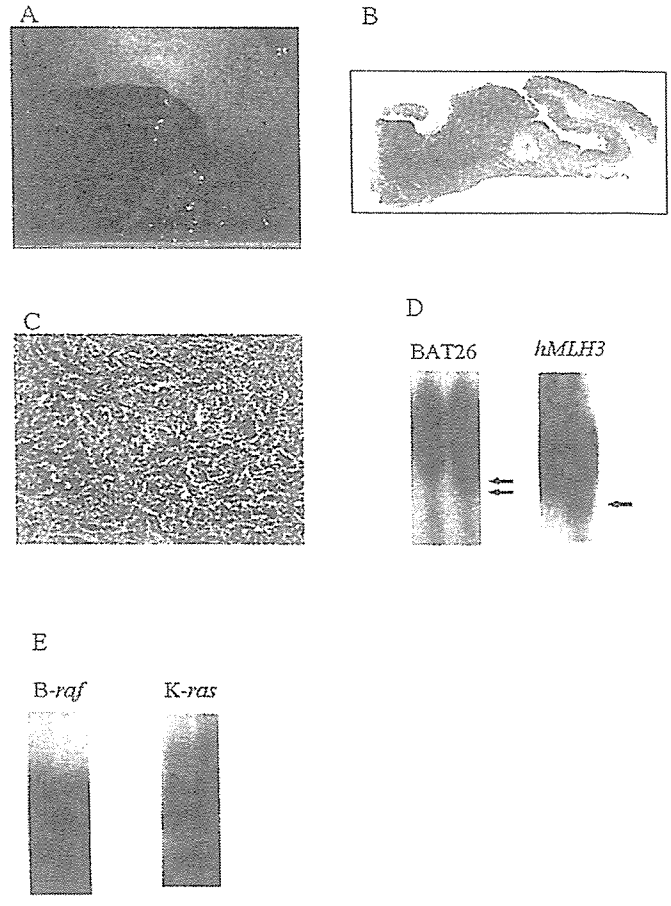

Figure 2. A representative case of diffuse-type gastric cancer. (A) Endoscopy revealed an ulcerated lesion at the posterior wall of the angular region. (B) Loupe appearance. (C) A histologically, diffuse, poorly differentiated adenocarcinoma (x200) was identified. (D) Microsatellite analysis showed alterations at BAT26 and *hMLH3*. (E) PCR-SSCP analysis showed no mobility shift of B-*raf* and K-*ras*.

genetic analyzer (Perkin-Elmer ABI, Foster City, CA). For the sequencing reaction, a PRISM AmpliTaq DNA polymerase FS ready reaction dye terminator sequencing kit (Perkin-Elmer ABI) was used.

Fisher's exact probability and Chi-square tests were used for comparisons of clinicopathologic and genetic factors. $P<0.05$ was regarded as significant.

Results

Representative cases are shown in Figs. 1 and 2. Clinicopathologic characteristics of young patients and older patients with gastric cancer are shown in Table II. The mean age of young gastric cancer patients was 35.3 (range, 18-40), and that of older gastric cancer patients was 64.5 (range, 44-90). Cancer in the whole of the stomach was found significantly more often in young patients than in older patients [11/53 (21%) vs. 1/66 (2%), respectively, P=0.0006 by Fisher's exact probability test]. Histopathologically, diffuse-type gastric cancer was found significantly more often in young patients than in older patients [52/68 (77%) vs. 21/66 (32%), respectively, P<0.0001 by Chi-square test]. Advanced tumors were significantly more prevalent in young patients than in older patients [40/65 (62%) vs. 26/63 (41%), respectively, P=0.022]. *H. pylori* infection was significantly less prevalent in young patients than in older patients [60/68 (88%) vs. 66/66 (100%), respectively, P=0.004]. Follicular gastritis was observed more frequently in young patients than in older patients [4/68 (6%) vs. 0/66 (0%), respectively, P=0.063 by Fisher's exact probability test].

Genetic characteristics of gastric cancers in young patients and in older patients are shown in Table III. MSI was found significantly more often in tumors in young patients than in tumors in older patients [10/65 (15%) vs. 2/51 (4%), respectively, P=0.040 by Fisher's exact probability test]. Mutations in *hMLH3* were found significantly more often in tumors in young patients than in tumors in older patients [5/56 (9%) vs. 0/51 (0%), respectively, P=0.036 by Fisher's exact probability test]. One detected K-*ras* mutation was a GGT to GCT (Gly to Ala) mutation at codon 12. Out of 10 B-*raf* mutations detected, 6 were CAT to CCG (His to Leu) mutations at codon 396 of exon 11, and 4 were CAG to CCG (Glu to Pro) mutations at codon 608 of exon 15. The frequencies of B-*raf* and K-*ras* mutations did not differ significantly between tumors in young patients and those in older patients.

The clinicopathologic characteristics of young gastric cancer patients are shown by sex in Table IV. There was a significant difference between the sexes in the number of tumors found in the lower-third of the stomach [2/22 females

Table III. Genetic characteristics of gastric cancer in young and older patients.

Genetic alteration	Young patients	Older patients	P-value
MSI			
+/-/NI	10/55/3 (15%)	2/49/15 (4%)	0.040
hMLH3 mutation			
+/-/NI	5/51/12 (9%)	0/51/15 (0%)	0.036
B-*raf* mutation			
+/-/NI	6/46/16 (12%)	4/46/16 (8%)	0.40
K-*ras* mutation			
+/-/NI	0/23/45 (0%)	1/32/33 (3%)	0.59

NI, not informative.

Table IV. Clinicopathologic characteristics of gastric cancer in young male and female patients.

Characteristics	Young male patients	Young female patients	P-value
Tumor location			
Lower third/others/NA	10/21/8	2/20/7	0.046
Histology			
Intestinal diffuse	11/28	5/24	0.22
Tumor depth			
Early/advanced/NA	15/21/3	10/19/0	0.55
Helicobacter pylori infection			
Positive/negative	34/5	26/3	0.53
Follicular gastritis			
Present/absent	2/37	2/27	0.57

NA, information not available.

Table V. Genetic characteristics of gastric cancer in young male and female patients.

Genetic alteration	Male patients	Female patients	P-value
MSI			
+/-/NI	2/36/1 (5%)	8/19/2 (30%)	0.010
hMLH3 mutation			
+/-/NI	2/30/7 (6%)	3/21/5 (13%)	0.36
B-*raf* mutation			
+/-/NI	4/28/7 (13%)	2/18/9 (10%)	0.58
K-*ras* mutation			
+/-/NI	0/13/26 (0%)	0/10/19 (0%)	-

NI, not informative.

(9%) vs. 10/31 males (33%), P=0.046 by Fisher's exact probability test].

The genetic characteristics of gastric cancer in young patients are shown by sex in Table V. MSI was found significantly more often in female patients than in male patients [8/27 (30%) vs. 2/38 (5%), respectively, P=0.010 by Fisher's exact probability test]. There were no significant differences in the frequencies of *hMLH3*, B-*raf*, and K-*ras* mutations between the sexes in young gastric cancer patients.

Discussion

Gastric cancers in young patients are reported to have different clinicopathologic characteristics from gastric cancers in older patients. For instance, diffuse-type gastric tumors and liver metastasis are reported to occur significantly more frequently in young patients than in older patients, and young patients are reported to have a more aggressive phenotype and poorer prognosis than older patients (8). These findings suggest that the pathways of gastric cancer in young patients differ from those in older patients. The increased frequency of cancer of the whole stomach and diffuse-type gastric cancer that we observed in young patients is similar to results reported previously. These data also suggest that the pathways of gastric cancer in young patients may differ from those in older patients.

We examined genetic alterations in gastric cancer to clarify whether the molecular profiles of tumors differ between young patients and older patients. Gastric cancer can occur as a hereditary non-polyposis colorectal cancer, whereby alterations in the mismatch repair genes (*hMLH1*, *hMSH2*, *hMSH6*, etc.) are responsible for colorectal, gastric, and endometrial tumor formation (13). Disrupted function of mismatch repair genes manifests as MSI and has been reported in 15-39% of sporadic gastric cancer. A single test of BAT26 can identify cases positive for high-level MSI (14,15). Several researchers have reported that MSI is rare (0-1.3%) in gastric cancer in young patients. However, Hayden *et al* (6) reported MSI in 6% of gastric cancers in young patients, and Semba *et al* (16) reported MSI in 22% of gastric cancers in young patients. In the present study, 15% of gastric cancers in young patients showed MSI, and MSI was found significantly more often in tumors in young patients than in tumors in older patients. In addition, tumors in young patients had significantly frequent mutations of *hMLH3*, one of the mismatch repair genes, compared with tumors in older patients. Mutation of the major mismatch repair genes, *hMSH2* and *hMLH1*, has not been detected in gastric cancers in young patients (17). Thus, it is possible that *hMLH3* mutation is a key genetic change in the development of gastric cancer in young patients. The number of cases examined in the present study was limited; further examination of genetic changes in a greater number of cases may be necessary.

We previously reported that follicular gastritis confers a high-risk for diffuse-type gastric cancer and is predominant in female patients (10,18). In the present study, cancer in the whole of the stomach and MSI were was found significantly more often in young females than in young males, and follicular gastritis tended to be more common in young females than in young males. These results suggest differences between the sexes in the genetic pathways of gastric cancer in young patients. This is the first reported study to focus on differences between the sexes regarding the clinicopathologic and genetic characteristics of gastric cancer in young patients.

ras genes are the most frequently mutated oncogenes in human cancers (19). The vast majority of *ras* mutations associated with human diseases involve K-*ras* (20). Activating point mutations of the gene affect codons 12 and 13. K-*ras* mutations are reported in 2.8-20% of gastric cancers (4). In the present study, K-*ras* mutations were detected in only 3% of tumors in older patients and in none of the tumors in young patients. The results were similar to those reported previously.

Recently, B-*raf* mutations have been reported in human malignancies, such as colon cancer and melanoma (21). Almost all reported B-*raf* mutations have occurred within two kinase domains (the G-loop domain and kinase domain), and the most common mutation is a single substitution, V599E. B-*raf* protein plays a central role in the ras/raf/mek/erk pathway, relaying signals from activated RAS proteins. B-*raf* mutations in gastric cancer are reportedly infrequent at 0-2.2% (22,23). In the present study, B-*raf* mutations were detected in 12% of tumors in young patients and in 8% of tumors in older patients. The percentages in the present study were high compared to those reported previously. However, there was no significant difference in the frequency of B-*raf* mutations between young patients and older patients.

The B-*raf* and K-*ras* mutations were infrequent, and there were no significant differences in the frequencies of these gene mutations between the sexes. These genes may not be key genetic alterations in the development of gastric cancers.

In conclusion, the clinicopathologic and genetic differences in gastric cancer that we observed between young patients and older patients suggest that the pathways of gastric cancer development differ between these two groups. In addition, the clinicopathologic and genetic differences that we observed between gastric cancer in young male patients and that in young female patients suggest different pathways. Thus, even among young patients, the pathways of gastric cancer development may differ, depending on sex.

References

1. Parkin DM, Pisani P and Ferlay J: Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 54: 594-606, 1993.
2. Meyers WC, Damiano RJ Jr, Rotolo FS and Postlethwait RW: Adenocarcinoma of the stomach. Changing patterns over the last 4 decades. Ann Surg 205: 1-8, 1987.
3. Miyoshi E, Haruma K, Hiyama T, Tanaka S, Yoshihara M, Shimamoto F and Chayama K: Microsatellite instability is a genetic marker for the development of multiple gastric cancers. Int J Cancer 95: 350-353, 2001.
4. Hiyama T, Haruma K, Kitadai Y, Masuda H, Miyamoto M, Tanaka S, Yoshihara M, Shimamoto F and Chayama K: K-*ras* mutation in *Helicobacter pylori*-associated chronic gastritis in patients with and without gastric cancer. Int J Cancer 97: 562-566, 2002.
5. Hiyama T, Tanaka S, Yoshihara M, Sasao S, Kose K, Shima H, Tuncel H, Ueno Y, Ito M, Kitadai Y, Yasui W, Haruma K and Chayama K: Chromosomal and microsatellite instability in sporadic gastric cancer. J Gastroenterol Hepatol 19: 756-760, 2004.
6. Hayden JD, Cawkwell L, Sue-Ling H, Johnston D, Dixon MF, Quirke P and Martin IG: Assessment of microsatellite alterations in young patients with gastric adenocarcinoma. Cancer 79: 684-687, 1997.
7. Mori M, Sugimachi K, Ohiwa T, Okamura T, Tamura S and Inokuchi K: Early gastric carcinoma in Japanese patients under 30 years of age. Br J Surg 72: 289-291, 1985.
8. Nakamura T, Yao T, Niho Y and Tsuneyoshi M: A clinicopathological study in young patients with gastric carcinoma. J Surg Oncol 71: 214-219, 1999.
9. Lauren P: The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. APMIS 64: 31-49, 1965.
10. Miyamoto M, Haruma K, Hiyama T, Kamada T, Masuda H, Shimamoto F, Inoue K and Chayama K: High incidence of B-cell monoclonality in follicular gastritis: a possible association between follicular gastritis and MALT lymphoma. Virchows Arch 440: 376-380, 2002.
11. Hiyama T, Tanaka S, Kitadai Y, Ito M, Sumii M, Yoshihara M, Shimamoto F, Haruma K and Chayama K: *p53* codon 72 polymorphism in gastric cancer susceptibility in patients with *Helicobacter pylori*-associated chronic gastritis. Int J Cancer 100: 304-308, 2002.
12. Akiyama Y, Nagasaki H, Nakajima T, Sakai H, Nomizu T and Yuasa Y: Infrequent frameshift mutations in the simple repeat sequences of *hMLH3* in hereditary nonpolyposis colorectal cancers. Jpn J Clin Oncol 31: 61-64, 2001.
13. Wu M, Semba S, Oue N, Ikehara N, Yasui W and Yokozaki H: BRAF/K-ras mutation, microsatellite instability, and promoter hypermethylation of hMLH1/MGMT in human gastric carcinomas. Gastric Cancer 7: 246-253, 2004.
14. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava S: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58: 5248-5257, 1998.
15. Hoang JM, Cottu PH, Thuille B, Salmon RJ, Thomas G and Hamelin R: BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. Cancer Res 57: 300-303, 1997.
16. Semba S, Yokozaki H, Yasui W and Tahara E: Frequent microsatellite instability and loss of heterozygosity in the region including BRCA1 (17q21) in young patients with gastric cancer. Int J Oncol 12: 1245-1251, 1998.
17. Lim S, Lee HS, Kim HS, Kim YI and Kim WH: Alteration of E-cadherin-mediated adhesion protein is common, but microsatellite instability is uncommon in young age gastric cancers. Histopathology 42: 128-136, 2003.
18. Miyamoto M, Haruma K, Yoshihara M, Sumioka M, Nishisaka T, Tanaka S, Inoue K and Chayama K: Five cases of nodular gastritis and gastric cancer : a possible association between nodular gastritis and gastric cancer. Dig Liver Dis 34: 819-820, 2002.
19. Kiaris H and Spandidos DA: Mutations of *ras* genes in human tumours (Review). Int J Oncol 7: 413-421, 1995.
20. Ellis CA and Clark G: The importance of being K-Ras. Cell Signal 12: 425-434, 2000.
21. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR and Futreal PA: Mutations of the BRAF gene in human cancer. Nature 417: 949-954, 2002.
22. Lee SH, Lee JW, Soung YH, Kim HS, Park WS, Kim SY, Lee JH, Park JY, Cho YG, Kim CJ, Nam SW, Kim SH, Lee JY and Yoo NJ: BRAF and KRAS mutations in stomach cancer. Oncogene 22: 6942-6945, 2003.
23. Oliveira C, Pinto M, Duval A, Brennetot C, Domingo E, Espin E, Armengol M, Hamelin R, Seruca R and Schwartz S Jr: BRAF mutations characterize colon but not gastric cancer with mismatch repair deficiency. Oncogene 22: 9192-9196, 2003.

Original Paper

Digestion

Digestion 2006;73:47–53
DOI: 10.1159/000092014

Received: November 15, 2005
Accepted: January 5, 2006
Published online: March 14, 2006

Development of a Novel Method to Detect *Helicobacter pylori cagA* Genotype from Paraffin-Embedded Materials: Comparison between Patients with Duodenal Ulcer and Gastric Cancer in Young Japanese

Hiroyuki Ueda[a] Masanori Ito[a] Hidetaka Eguchi[b] Shinji Tanaka[c] Masaharu Yoshihara[d] Ken Haruma[e] Masanori Hatakeyama[f] Kazuaki Chayama[a]

[a]Department of Medicine and Molecular Science, Hiroshima University, [b]Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, [c]Department of Endoscopy, Hiroshima University Hospital, Hiroshima; [d]Health Service Center, Hiroshima University, Higashi-Hiroshima; [e]Gastroenterology Unit, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, and [f]Division of Molecular Oncology, Institute for Genetic Medicine, Graduate School of Science, Hokkaido University, Sapporo, Japan

Key Words

Helicobacter pylori · *cagA* gene · Gastric cancer · Duodenal ulcer · Paraffin-embedded section

Abstract

Background/Aim: *cagA* gene polymorphism of *Helicobacter pylori* contributes to clinical outcome of patients. We investigated the implication of the *cagA* polymorphism in young Japanese patients using paraffin-embedded sections. ***Methods:*** We studied 71 young patients with gastric cancer or with duodenal ulcer. *H. pylori* infection was confirmed by sections with Giemsa staining and immunohistochemical staining and the degree of gastritis was evaluated. DNA was extracted from paraffin-embedded sections of 20 patients both from the gastric corpus and the antrum. A portion of *cagA* gene was amplified with polymerase chain reaction, followed by direct sequencing of the fragment. ***Results:*** We established a novel method to determine the *cagA* subtype using paraffin-embedded sections. We found that all the samples possessed East-Asian type *cagA* both in the corpus and the antrum, not only in patients with gastric cancer but also with duodenal ulcer. Although the *cagA* gene sequence was completely identical between the gastric corpus and the antrum in all patients, the corpus gastritis was more prominent in patients with gastric cancer than those with duodenal ulcer. ***Conclusions:*** *cagA* polymorphism can be evaluated with the use of paraffin-embedded sections. The degree of corpus gastritis may not be regulated by *cagA* diversity only.

Introduction

Helicobacter pylori infection is a critical factor for promoting atrophic gastritis [1]. Long-term infection of *H. pylori* results in glandular atrophy and intestinal metaplasia. Since corpus atrophic gastritis is a fundamental and essential status for human gastric carcinogenesis, *H. pylori* is regarded as an important carcinogen in the devel-

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0012–2823/06/0731–0047$23.50/0

Accessible online at:
www.karger.com/dig

Masanori Ito, MD, PhD
Department of Medicine and Molecular Science
Hiroshima University, Hiroshima 734-8551 (Japan)
Tel. +81 82 257 5191, Fax +81 82 257 5194
E-Mail maito@hiroshima-u.ac.jp

opment of human gastric cancer [2]. Indeed, it has been accepted that there is a strong association between *H. pylori*-associated gastritis and gastric cancer [3–6]. A clinical study by Uemura et al. [8] confirmed a strong association between the occurrence of gastric cancer and *H. pylori* infection followed by corpus gastritis. Atrophic gastritis and gastric cancer are very common diseases in Japan, since the high prevalence of *H. pylori* infection has especially been confirmed in elderly people [9]. However, not all patients with *H. pylori* infection have gastric cancer, so it is clinically important to select the population with a high risk for gastric cancer development [10].

H. pylori cagA protein is an important virulent factor for gastric mucosa injury. Huang et al. [11] demonstrated the strong association between anti-cagA seropositivity and development of gastric cancer, suggesting an importance of cagA for gastric carcinogenesis. Recent studies have clarified the molecular mechanism of gastric mucosal injury induced by cagA protein. cagA protein produced in the bacterial cell is translocated into the host cell by the type IV secretory system, followed by tyrosine phosphorylation by src-family kinases and activation of SHP-2 phosphatase [12, 13]. Recent studies revealed that cagA protein showed diversity and was subclassified into two types, namely Western type and East-Asian type. The latter type was reported to have a high affinity to SHP-2 and was thus regarded as a more harmful form than the Western type [14]. In fact, diversity of cagA protein was assessed and showed a tight relationship between its diversity and the clinical outcome [15].

However, until now, most studies have been performed with the use of isolated colonies, resulting in the uncertainty of whether it really reflects the original character of *H. pylori* in the stomach. In addition, it is clinically important to investigate the topography of gastric inflammation, which may be influenced by the heterogeneous distribution of *H. pylori*. Then, in the present study, we investigated the cagA subtype of *H. pylori* with the use of DNA samples extracted from paraffin-embedded sections that reflect the original character of the bacteria. Moreover, we studied the diversity in the samples from the gastric corpus and the antrum separately to discuss the heterogeneity of the cagA subtype in the stomach.

Methods

Patients

We studied 32 patients with gastric cancer (20 men, mean age 26.6 years; 12 women, mean age 25.6 years) and 39 patients with duodenal ulcer (36 men, mean age 25.1 years; 3 women, mean age 25.6 years). All patients were <30 years and underwent gastrectomy. The resected stomach was fixed in buffered formalin and embedded in a paraffin block. Except for the pathological lesions, non-neoplastic gastric mucosae in the lesser curvature were cut and embedded in paraffin sections in the same manner. These non-neoplastic mucosas were used for the present examinations. All patients had histological gastritis either in the gastric corpus and antrum, and were confirmed as being *H. pylori*-positive by Giemsa staining. Patients who received eradication therapy were not included in this study. We obtained informed consent from all patients and the Ethical Committee of Hiroshima University approved our protocol.

Evaluation of Histology of Gastritis

In each patient, histological gastritis of the lesser curvature of the corpus and antrum were evaluated with the use of the sections stained with hematoxylin and eosin (HE). We scored the degree of gastritis (mononuclear infiltration and activity; from 0 to 3) with the criteria of the updated Sydney System [16]. Two specialists (M.I. and K.H.) who independently scored the degree of gastritis were blind to the clinical information.

DNA Extraction from Paraffin-Embedded Sections

We randomly selected 20 patients (10 with gastric cancer and 10 with duodenal ulcer) and conducted DNA extraction from the tissue sections of the patients. Tissue sections of 4 μm in thickness were placed on glass slides and stained with HE. The tissue sections were then dehydrated in graded ethanol solutions and dried without a cover glass. Tissues were scraped from the slides with sterile needles. DNA was extracted from the tissues by incubation in 20 μl of extraction buffer (100 m*M* Tris-HCl; 2 m*M* EDTA, pH 8.0; 400 μg/μl proteinase K) at 55°C overnight. The tubes were boiled for 7 min to inactivate the proteinase K, and then 2 μl of the extracts was used for each polymerase chain reaction (PCR) amplification.

PCR Reaction

To determine the cagA subtype, two pairs of primers (forward: cagA01 and reverse: cagA02 or cagA05, respectively) were used for PCR amplification, yielding 117- and 92-basepair (bp) products, respectively (table 1). Each 20 μl of reaction mixture contained 0.2 μl of Pyrobest DNA polymerase (5 units/μl; Takara, Shiga, Japan), 2 μl of 10× Pyrobest buffer II, 2 μl of dNTP mixture (2.5 m*M* each), and 1 μl each of forward and reverse primers. The reaction mixtures were heated to 95°C for 5 min, followed by 50 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30 s, and elongation at 72°C for 30 s. After PCR, the products were electrophoresed on 8% polyacrylamide gels containing 1× TBE buffer (50 m*M* Trizma base, 67 m*M* borate, 1 m*M* EDTA).

Sequencing

DNA bands were excised from the gels and the DNAs were eluted and purified using QIAquick Gel Extraction kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Then, purified DNA fragments were subjected to sequence reaction using BigDye Terminators Version 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA) and ABI Prism 310 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instruction.

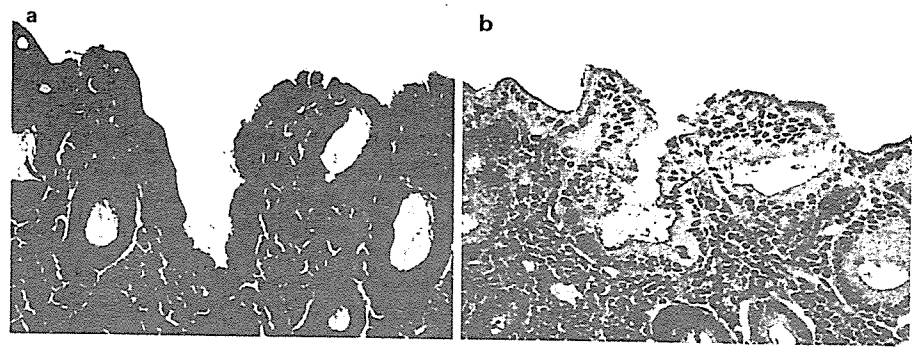

Fig. 1. Detection of *H. pylori* in the gastric mucosa from the corpus by HE section (**a**) and by immunohistochemical staining (**b**) in a 26-year-old male patient. Arrow indicates the bacterium just on the epithelial cells.

Table 1. Histology in the corpus and antrum of the patient groups

	Corpus			Antrum		
	GC (n = 30)	DU (n = 36)	p	GC (n = 32)	DU (n = 39)	p
Infiltration						
Grade 0 or 1, n (%)	10 (33.3)	32 (88.9)		8 (25.0)	6 (15.4)	
Grade 2 or 3, n (%)	20 (66.7)	4 (11.1)	<0.001	24 (75.0)	33 (84.6)	>0.3
Activity						
Grade 0 or 1, n (%)	20 (66.7)	35 (97.2)		19 (59.4)	20 (51.3)	
Grade 2 or 3, n (%)	10 (33.3)	1 (2.8)	0.002	13 (40.6)	19 (48.7)	>0.4

Immunohistochemistry

Four-micrometer sections of formalin-fixed paraffin-embedded tissues were used for immunohistochemical staining. After deparaffinization and hydration, internal peroxidase was blocked by incubation with 0.3% H_2O_2 in methanol for 15 min. After incubation with 5% skim milk/PBS for 20 min, the sections were reacted with the primary antibody (diluted with PBS) for 2 h at room temperature. The primary antibody used was anti-*H. pylori* polyclonal antibody (dilution of 1:50; Dako, Kyoto, Japan). Antigen retrieval was carried out with microwave treatment before reacting with primary antibody.

Statistics

Statistical analysis was performed by χ^2 test and Fisher's exact test with SPSS Version 11.5J software (SPSS Inc., Chicago, Ill., USA). A p value of <0.05 was considered statistically significant.

Results

Immunohistochemical Detection of H. pylori

We examined the *H. pylori* status by immunohistochemical staining. As shown in figure 1, we could hardly detect the bacteria in HE sections. In most sections the mucous layer over the mucosal epithelium had been washed out and could not be found in the sections. In immunohistochemical analysis, several bacteria could be detected just on the epithelium. The image is not different between sections from the two groups (gastric cancer and duodenal ulcer).

Comparison of Histology in Gastritis between Gastric Cancer and Duodenal Ulcer Patients

First, we compared the grades of gastritis between sections from young patients with gastric cancer and with duodenal ulcer. As shown in table 1, the degree of antral gastritis was not statistically different in neutrophil activity and chronic inflammation between these two groups. On the other hand, in the gastric corpus the degree of gastritis was statistically more prominent (activity, $p = 0.002$, and chronic inflammation, $p < 0.001$) in sections with gastric cancer than in those with duodenal ulcer.

Establishment of Amplification Method of cagA *Gene Using Paraffin-Embedded Sections*

Since DNA samples were degraded in various degrees and the amount of *H. pylori* DNA relative to the human DNA was small, many experimental improvements

cagA Analysis from Paraffin-Embedded Section

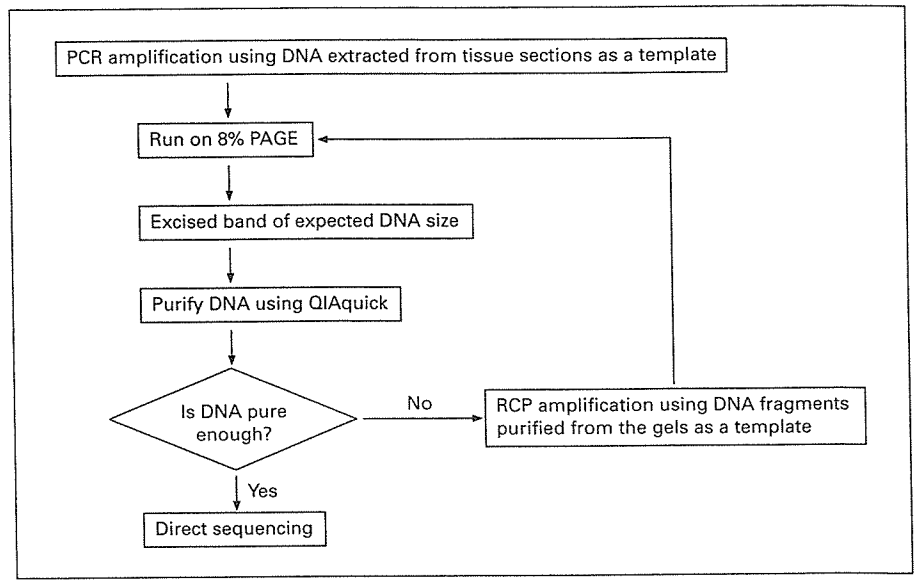

Fig. 2. The method from PCR amplification to direct sequence.

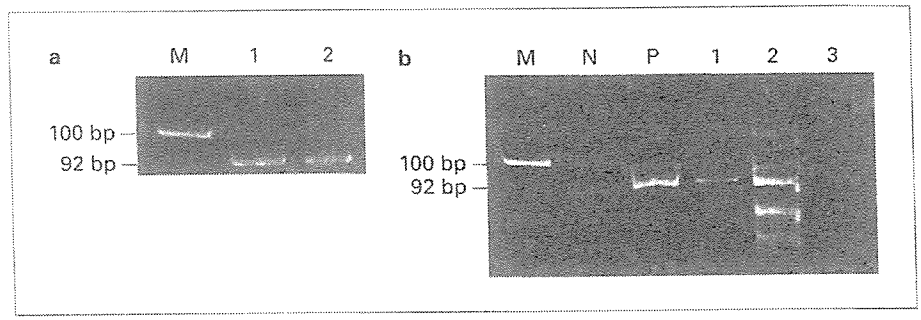

Fig. 3. Detection of PCR products. **a** Products with single amplification (lane 1) and those by repeated PCR three times (lane 2). **b** Final products of amplified *H. pylori* DNA from 3 patients (lanes 1–3). M = Marker; N = negative control; P = positive control.

Table 2. Oligonucleotides used to detect the CagA

Gene	Primer	Primer sequence	Size, bp
cagA	cagA01	5′-TAGCCCTGAACCCATTTACG-3′	(01-02) 117
	cagA02	5′-TGTTCCCTTGAAAGCCCTAC-3′	
	cagA05	5′-TGAGATCACTAACTGCAGCAC-3′	(01-05) 92

were needed to obtain PCR products of good quality capable for conducting direct sequence reaction (fig. 2). First, we found that PCR products should not exceed 100 bp in size to obtain reproducible amplification. Primers that were used in this study are shown in table 2. For the PCR amplification, Pyrobest DNA polymerase possessing proofreading activity was suitable for our experiments. Since the first PCR products contained additional DNA fragments (fig. 3a, lane 1), the amplified fragments were separated and purified from the 8% PAGE and the purified DNA fragments were used as a template for second or third PCR to obtain a single band of *cagA* fragment (fig. 3a, lane 2). In order to confirm that obtained PCR fragments were specific for *cagA*, we always included negative controls in each reaction (fig. 3b). As for the reproducibility of this method, we confirmed that we could obtain completely identical results by repeating the examination with the same section (data not shown).

Comparison of cagA Subtype between Gastric Cancer and Duodenal Ulcer Patients

Using direct sequencing, we were able to obtain the *cagA* DNA sequences from all the tested samples. The

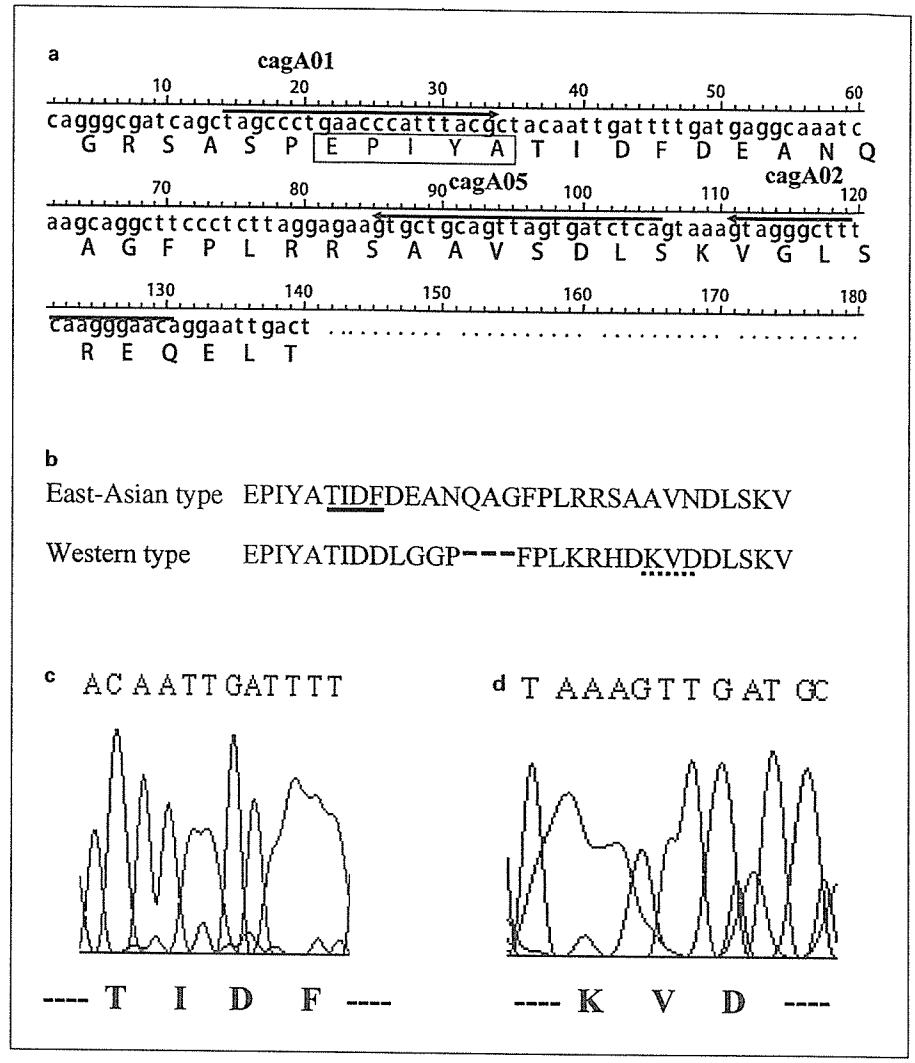

Fig. 4. a The information about the place of primers (cagA 01, 02 and 05). **b** Representative amino acid sequence of East-Asian and Western type cagA protein [from 14]. Results of direct sequence of DNA extracted from paraffin-embedded section with the use of primers cagA01-02 (**c**, **d**). The results of direct sequence of *cag*A DNA (upper) and encoding amino acids cagA protein (lower) were demonstrated. **c** The sequence of East-Asian cagA (#974–977, +2 to +5 from Y; black line in **b**). **d** The sequence of Western cagA (+17 to +19 from Y; dotted line in **b**). The patient used in figure 4d was not included in our study.

information about the place of primers used in our study is demonstrated in figure 4a, and typical amino acid sequences of two cagA subtypes are also demonstrated in figure 4b. The representative results are shown in figure 4c. In gastric cancer patients, we detected only East-Asian type cagA both in the gastric corpus and antrum. In duodenal ulcer patients, the same results were obtained not only in the gastric corpus but also in the antrum. Although we detected only East-Asian type cagA in the present study, we confirmed that the Western type cagA could be detected in another examination and denied the possibility that our system could only be applicable for detecting East-Asian type cagA (fig. 4d).

cagA Subtype Heterogeneity between the Gastric Antrum and the Corpus

Then, we compared all the DNA sequences encoding amino acids of cagA protein between those from the gastric corpus and from the antrum. The DNA sequence was completely identical between the DNAs extracted from the different sites.

Discussion

East-Asian cagA is supposed to be more virulent to epithelial injury and is regarded as being more carcinogenic for gastric mucosa than the Western type. In fact, the international distribution of the gastric cancer incidence could be explained by the diversity of this harmful

cagA Analysis from Paraffin-Embedded Section

type of cagA [17]. However, in Japanese patients, almost all bacteria had cagA protein and have revealed that its subtype was the East-Asian type [18, 19]. Therefore, it is difficult to explain the difference in clinical outcome induced by *H. pylori* infection within Japanese patients only by the diversity of cagA status.

Focus must be placed on the methodology of these studies. In most studies, samples were extracted from isolated colonies of *H. pylori*. In the status, it is controversial whether the biological characters of isolated colonies reflect the original character of the bacteria in vivo, since the presence of metastability of the *H. pylori* could not be completely denied [20]. Another important problem is the topography of gastric inflammation. In gastric carcinogenesis, corpus-predominant gastritis showed a tighter relationship with gastric cancer, whereas antral-predominant gastritis is a negative factor for gastric carcinogenesis [8]. It is difficult to explain the heterogeneous distribution of gastritis inflammation only from the difference of solitary bacteria.

In the present study, we examined and compared the difference in the status of gastritis between two groups: the first included gastric cancer patients <30 years whose *H. pylori* was expected to be virulent in gastric carcinogenesis and the other included duodenal ulcer patients who were considered to have *H. pylori* that is less potential in gastric carcinogenesis. We have previously reported the tight association between *H. pylori* infection and the occurrence of gastric cancer in young patients [21, 22]. We then compared the status of *H. pylori* cagA status in these two groups, and the improved points in this study were (1) to use the paraffin-embedded section to avoid the metastability of the bacteria, and (2) to examine the status of gastric corpus and antrum separately in each patient.

In the present study, we could confirm that corpus gastritis was more prominent in gastric cancer patients than in duodenal ulcer patients, whereas antral gastritis was not different between these two groups. These findings are completely compatible with the report by Uemura et al. [8]. However, unexpectedly, the cagA status was not different between these two groups not only in the gastric antrum but also in the gastric corpus. This suggests that the status of gastritis, especially in the gastric corpus, is not affected by the status of the cagA protein in Japanese subjects. The main cause is still unclear but this might be regulated in the step of bacterial adhesion or of intracellular signaling after SHP-2 activation. A previous report has demonstrated that intragastric status including acid secretion may be a key factor for the mechanism of corpus inflammation induced by the cagA infusion system [23], but some controversial results have also been published. It is still controversial whether *H. pylori* in the stomach is monotonous or heterogeneous. We previously studied the bacterial resistance for antibiotics and demonstrated that the heterogeneity of *H. pylori* could be found in approximately 30% of the patients [24]. In the present study, the sequence of the cagA hot spot is completely identical between gastric corpus and the antrum in all cases examined. It is unlikely that the heterogeneous distribution as for cagA subtype could be detected in Japanese patients. Differing from the drug resistance gene, *cagA* gene may be highly conserved and not be under the status of easy metastability.

The main advantage in this study is the establishment of a methodology to examine the cagA status using paraffin-embedded sections. Fortunately, by using the paraffin-embedded surgical sections, we succeeded in reducing the influence of *H. pylori* floating in the gastric mucous. Previously, only cagA status (positive or negative) was determined by using paraffin-embedded sections, and no report could be found as for the direct sequencing of *cagA* gene [25, 26]. Our method seems to be time- and money-consuming comparing the analysis with the use of fresh biopsy specimens. However, in old and rare cases, such as gastric carcinoma in young patients, often we only have to use the paraffin-embedded sections as a starting material. This is the main purpose of our study and we believe our method may contribute to the further extension in this field including retrospective studies using old samples.

However, our protocol may be laborious since it requires the repeated PCR reactions to obtain a high-quality result. For example, as in our experience, the size of the PCR product was limited to 100 bp in length and three times repeated PCR was needed for most cases. In addition, our experience showed that the results were affected by the conditions of sample preparation including the fixation procedure. An immediate fixation after resection may be important for the maintenance of the good quality of *H. pylori* DNA, and the use of buffered formalin seemed to be essential. Long-term washing of fixed samples may result in extreme reduction of bacterial number and should be avoided. Further technical improvement of our method may be required for application to the clinical examination in practice. In the next step, it may be possible to distinguish two types of *cagA* gene without sequencing. Actually, the size of the PCR product in each subtype was different (117 vs. 108 bp) when we used cagA 01-02 primers. As demonstrated in figure 3, we found

some additional bands and these made it difficult to distinguish the cagA pattern only by evaluating the pattern after electrophoresis. However, it is important to try to make better primers for this purpose.

Taken together, this is the first report demonstrating the cagA status of gastric cancer and duodenal ulcer in young patients using paraffin-embedded sections. Our results will provide the next strategy to clarify the difference in corpus gastritis between these patients. The clarification of bacterial adhesion including the intragastric condition may be a next step in this matter.

Acknowledgements

The authors thank Dr. Kei Nakachi (Radiation Effects Research Foundation, Hiroshima, Japan) and Drs. Kenichi Imagawa and Yosuke Harada (Theranostics Research Center Otsuka Pharm. Co. Ltd, Tokushima, Japan) for the invaluable advice for our experiments. Also, we thank Dr. Chiaki Inokuchi (Inokuchi Hospital Higashi-Hiroshima, Japan) for her kind supply of surgically resected specimens.

References

1 Kawaguchi H, Haruma K, Komoto K, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* infection is the major risk factor for atrophic gastritis. Am J Gastroenterol 1996;91:959–962.

2 Correa P: *Helicobacter pylori* and gastric carcinogenesis. Am J Surg Pathol 1995;19(suppl 1):S37–S43.

3 Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M: *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. Gastroenterology 1998;115:642–648.

4 Sugiyama A, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M: *Helicobacter pylori* infection enhances *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res 1998;58:2067–2069.

5 Huang JQ, Sridhar S, Chen Y, Hunt RH: Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology 1998;114:1169–1179.

6 Komoto K, Haruma K, Kamada T, Tanaka S, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* infection and gastric neoplasia: correlations with histological gastritis and tumor histology. Am J Gastroenterol 1998;93:1271–1276.

7 International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, Liver Flukes, and *Helicobacter pylori:* Lyon, France. International Agency for Research on Cancer; 1994:177–240.

8 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784–789.

9 Asaka M, Kimura T, Kudo M, Takeda H, Mitani S, Miyazaki T, Miki K, Graham DY: Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. Gastroenterology 1992;102:760–766.

10 Ito M, Tanaka S, Kamada T, Haruma K, Chayama K: Causal role of *Helicobacter pylori* infection and eradication therapy in gastric carcinogenesis. World J Gastroenterol 2006 (in press).

11 Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH: Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology 2003;125:1636–1644.

12 Asahi M, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C: *Helicobacter pylori* cagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 2000;191:593–602.

13 Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M: SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* cagA protein. Science 2002;295:683–686.

14 Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M: Biological activity of the *Helicobacter pylori* virulence factor cagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 2002;99:14428–14433.

15 Azuma T, Yamazaki S, Yamakawa A, Ohtani M, Muramatsu A, Suto H, Ito Y, Dojo M, Yamazaki Y, Kuriyama M, Keida Y, Higashi H, Hatakeyama M: Association between diversity in the Src homology 2 domain-containing tyrosine phosphatase binding site of *Helicobacter pylori* cagA protein and gastric atrophy and cancer. J Infect Dis 2004;189:820–827.

16 Dixon MF, Genta RM, Yardley JH, Correa P: Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–1181.

17 Hatakeyama M: Oncogenic mechanisms of the *Helicobacter pylori* cagA protein. Nat Rev Cancer 2004;4:688–694.

18 Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, Shiratori Y, Omata M: Structure of *cag* pathogenicity island in Japanese *Helicobacter pylori* isolates. Gut 1999;44:336–341.

19 Azuma T, Yamakawa A, Yamazaki S, Ohtani M, Ito Y, Muramatsu A, Suto H, Yamazaki Y, Keida Y, Higashi H, Hatakeyama M: Distinct diversity of the *cag* pathogenicity island among *Helicobacter pylori* strains in Japan. J Clin Microbiol 2004;42:2508–2517.

20 Backstrom A, Lundberg C, Kersulyte D, Berg DE, Boren T, Arnqvist A: Metastability of *Helicobacter pylori* bab adhesin genes and dynamics in Lewis b antigen binding. Proc Natl Acad Sci USA 2004;101:16923–16928.

21 Haruma K, Komoto K, Kamada T, Ito M, Kitadai Y, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* is a major risk factor for gastric carcinoma in young patients. Scand J Gastroenterol 2000;35:255–259.

22 Ito M, Haruma K, Kamada T, Kitadai Y, Hidaka T, Tsuda T, Komatsu H, Fukuhara T, Yoshihara M, Chayama K: Reduction in the incidence of *Helicobacter pylori*-associated carcinoma in Japanese young adults. Oncol Rep 2001;8:633–636.

23 Merrell DS, Goodrich ML, Otto G, Tompkins LS, Falkow S: pH-regulated gene expression of the gastric pathogen *Helicobacter pylori*. Infect Immun 2003;71:3529–3539.

24 Masuda H, Hiyama T, Yoshihara M, Tanaka S, Shimamoto F, Haruma K, Chayama K: Necessity of multiple gastric biopsies from different sites for detection of clarithromycin-resistant *Helicobacter pylori* strains. Scand J Gastroenterol 2003;38:942–946.

25 Scholte GH, van Doorn LJ, Cats A, Bloemena E, Lindeman J, Quint WG, Meuwissen SG, Kuipers EJ: Genotyping of *Helicobacter pylori* in paraffin-embedded gastric biopsy specimens: relation to histological parameters and effects on therapy. Am J Gastroenterol 2002;97:1687–1695.

26 Koehler CI, Mues MB, Dienes HP, Kriegsmann J, Schirmacher P, Odenthal M: *Helicobacter pylori* genotyping in gastric adenocarcinoma and MALT lymphoma by multiplex PCR analyses of paraffin wax embedded tissues. Mol Pathol 2003;56:36–42.

Journal of Gastroenterology and Hepatology (2005) **20**, 1241–1245 DOI: 10.1111/j.1400-1746.2005.03928.x

GASTROENTEROLOGY

A combination of the *Helicobacter pylori* stool antigen test and urea breath test is useful for clinical evaluation of eradication therapy: A multicenter study

MASANORI ITO,* SHINJI TANAKA† SUNJIN KIM,*,‡ KAZUMASA TAHARA,‡ YUZURU KAWAMURA,‡ MASAHARU SUMII,§ YOSHIHIKO TAKEHARA,¶ KOZO HAYASHI,** EIICHI OKAMOTO,†† MASAKI KUNIHIRO,†† TOSHIRO KUNITA,†† SHINOBU IMAGAWA,* SHUNSUKE TAKATA,* HIROYUKI UEDA,* YASUO EGI,* TORU HIYAMA,‡‡ YOSHITAKA UENO,* YASUHIKO KITADAI,* MASAHARU YOSHIHARA‡‡ AND KAZUAKI CHAYAMA*

**Department of Medicine and Molecular Science, Hirsohima University, †Department of Endoscopy, Hiroshima University Hospital, ‡Kawamura Hospital, §Hiroshima Kinen Hospital, Hiroshima, ¶Saiseikai Kure Hospital, Kure, **Hiroshima Kampo Health Planning and Medical Center, ††Chuden Hospital, Hiroshima and ‡‡Health Service Center, Hiroshima University, Higashi-Hiroshima, Japan*

Abstract

Background: *Helicobacter pylori* stool antigen (HpSA) test is a new tool for evaluating the *H. pylori* infection. The present study was carried out to investigate the clinical usefulness of the HpSA test in the evaluation of eradication therapy by comparing it with the ^{13}C-urea breath test (UBT).

Methods: One hundred and five patients received eradication therapy for *H. pylori*. After more than 8 weeks, the success of the therapy was evaluated by the HpSA test and the UBT. Concordant results were regarded as a final diagnosis, but when the results were discordant, histological examination was carried out.

Results: Of the 105 patients receiving eradication therapy for *H. pylori*, 25 patients were regarded as *H. pylori* positive by the UBT and and 20 patients were regarded as *H. pylori* positive by the the HpSA test. Nine patients (8.6%) showed discordant results (seven cases with UBT(+) and HpSA(–), and two with UBT(–) and HpSA(+)). Five cases out of nine were ultimately judged as having a false-positive result of the UBT, and in these cases the UBT values were relatively low (below 10 per thousand). The final diagnostic accuracies of the UBT and the HpSA test were 94.3% (88.0–97.9%; 95% CI) and 97.1% (91.9–99.4%), respectively. When we used the HpSA test in cases with weakly positive UBT values, we were able to diagnose the correct status of *H. pylori* infection after eradication in 99% of all patients (94.8–100.0%).

Conclusion: The HpSA test is a useful tool for the evaluation of eradication therapy and a combination of the HpSA test and UBT is clinically recommended.

Key words: eradication therapy, *Helicobacter pylori*, *Helicobacter pylori* stool antigen, ^{13}C-urea breath test.

INTRODUCTION

Helicobacter pylori eradication is a popular therapy for peptic ulcer patients in Japan. Successful eradication therapy should result in the reduction of ulcer recurrence in Japanese patients.[1] Eradication therapy has come to be used for many gastric diseases including mucosa-associated lymphoid tissue (MALT) lymphoma (MALToma).[2] Although the prevalence of infection is gradually decreasing, the clinical application and

Correspondence: Dr Masanori Ito, Department of Medicine and Molecular Science, Hiroshima University, 1-2-3 Kasumi Minami-ku Hiroshima, 734-8551, Japan. Email: maito@hiroshima-u.ac.jp

Accepted for publication 11 September 2004.

importance of this therapy will increase in the near future.

To evaluate the final outcome of eradication therapy, many kinds of examination techniques are applicable. Although non-invasive methods are favorable for most patients, the serological test for anti-*H. pylori* antibodies is not suitable because it takes a long-time to be normalized. It is likely that the ^{13}C-urea breath test (UBT) will become the most popular and powerful method to examine the results of eradication therapy. However, the question has been raised by some researchers as to what is the most appropriate cut-off value (2.5 or 3.5 per thousand).[3] In addition, the high cost compared with the other methods is a problem.

Recently, the *H. pylori* stool antigen (HpSA) test was established and came to be applied in clinical situations. The Maastrichet Report described the equal usefulness of the UBT and the HpSA test for the judgment of not only *H. pylori* infection but also of the success of eradication therapy.[4] In the present study, we investigated the clinical usefulness of the HpSA test for the evaluation of the success of the eradication therapy by comparing it with the UBT.

METHODS

Patients

A total of 105 patients with *H. pylori* infection (55 men, mean age 56.6 years; 65 with peptic ulcers, five with gastric MALToma, and 35 with dyspepsia) were enrolled in the present study from August 2002 to December 2003, and all gave informed consent. The ethical committee of Hiroshima University approved the protocol. All patients received a gastroscopy or an X-ray examination of the upper gastrointestinal series and were confirmed to have no serious diseases present in the stomach. No patients who had received antibiotics or proton-pump inhibitors prior to entry were included in the study. *H. pylori* infection was examined by the use of a least one of three methods; the rapid urease test, ^{13}C-urea breath test or the presence of serum IgG antibodies against *H. pylori* (E-plate, Eiken, Tokyo, Japan).

An evaluation of eradication therapy was carried out at least 8 weeks after completion of therapy that involved the administration of lansoprazole (60 mg, b.i.d.), amoxicillin (1500 mg, b.i.d.) and clarithromycin (400 or 800 mg, b.i.d.).

Helicobacter pylori stool antigen and ^{13}C-urea breath test

In all patients, the *H. pylori* stool antigen test (HpSA, Meridian Diagnostics, Cincinnati, OH, USA)[5] and ^{13}C-urea breath test (UBT; Ubit IR-200, Otsuka, Tokushima, Japan)[6] were routinely carried out.

According to the manufacturer's instructions, a pieces of fecal samples were diluted in the sample solution, and stored at room temperature until analyzed. The HpSA test used polyclonal anti-*H. pylori* antibodies absorbed to microwells as a capture antibody. Sample solution was added and followed by the reaction with enzyme-conjugated polyclonal antibody. After addition of a substrate solution, the results were read by spectrophotometry at 450/630 nm.

UBT test was carried out as previously reported.[6] Fasting patients drank 100 mg of ^{13}C urea powder with 100 mL of tap water while they were in the sitting position and then immediately rinsed their mouths with water to remove the residual drug. Patients were placed in the left lateral decubitus position for 5 min and then in the sitting position for 15 min. Breath samples were collected before the drug was given, and then 20 min after the drug was given.

The cut-off values for the HpSA test and the UBT were 0.120 (OD 450/630 nm)[5] and 2.5 per thousand (delta-$^{13}CO_2$),[6] respectively. When the HpSA test value was $\geq$0.100 and <0.120, the HpSA test was re-examined. In 13 cases where the UBT values were <10.0, the delta-$^{13}CO_2$ was measured not only by infrared spectrometer but also by mass spectrometry analysis. If the results of the HpSA and UBT were identical, the result was regarded as the final decision. When we had discordant results, the following histological examination was carried out to make the final decision.

Histological examination

Five biopsy specimens were taken (two from the antrum, two from the corpus and one from gastric angle) and *H. pylori* colonization and grades of gastritis were evaluated by the criteria of the updated Sydney system.[7] Specimens were stained with hematoxylin and eosin (HE) and Giemsa staining. Specimens were regarded as *H. pylori* negative when no bacteria was identified and active inflammation was absent in both specimens.[8] Two experts (MI and SK) judged the histological gastritis independently without clinical information on the patients.

RESULTS

Helicobacter pylori stool antigen and ^{13}C-urea breath test

Correlation of the UBT and the HpSA test are shown as dot plots in Figure 1. Out of all 105 patients enrolled, positive results were obtained from 25 and 20 patients with the use of the UBT and the HpSA test, respectively. Seventy-eight and 18 patients were simultaneously negative and positive by the two methods, respectively. Nine patients (8.6%) showed discordant results; seven showed UBT(+) and HpSA(–) and two showed UBT(–) and HpSA(+) (Table 1). We evaluated the UBT value using two different methods (infrared spectrometry and mass spectrometry) and confirmed the very close relationship between the two results ($r = 0.995$, $P < 0.0001$; $n = 18$).

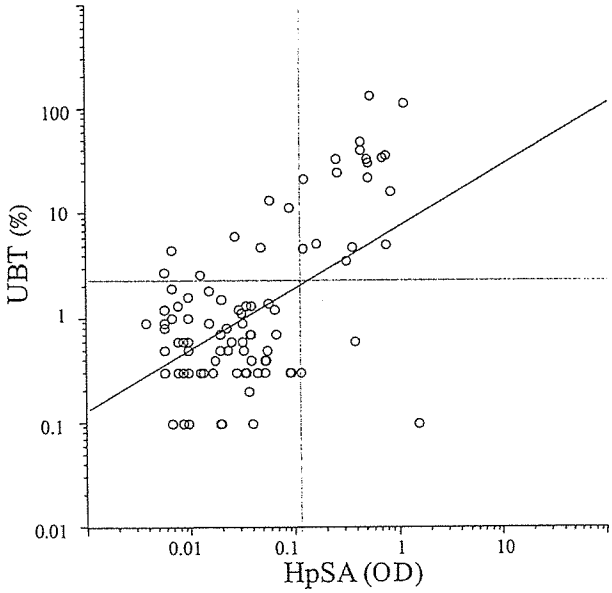

Figure 1 Correlation between the ^{13}C-urea breath test (UBT) and the *Helicobacter pylori* stool antigen (HpSA) test in patients who have undergone eradication therapy. Dot-line indicates the cut-off value in each examination. Fit line; log (UBT) = 0.586 × log (HpSA) + 0.861, $r = 0.506$, $P < 0.0001$.

Table 1 Results of ^{13}C-urea breath test and *Helicobacter pylori* stool antigen test in patients who have undergone eradication therapy

	Evaluation by HpSA		
	Negative	Positive	Total
Evaluation by UBT			
Negative	78	2	80
Positive	7	18	25
Total	85	20	105

HpSA, *Helicobacter pylori* stool antigen test; UBT, ^{13}C-urea breath test.

Histological examination in patients with discordant results

In nine cases where there were discordant results, a histological examination was carried out with gastric biopsy specimens. Results are summarized in Table 2. Histological evaluation showed results concordant with UBT test in three cases and with the HpSA test in six cases. In cases with a false positive reaction with the UBT, the delta-$^{13}CO_2$ level was relatively low (below 10 per thousand).

The diagnostic accuracy of the UBT and the HpSA test was 94.3% (88.0–97.9%; 95% CI) and 97.1% (91.9–99.4%), respectively. When we used the HpSA test in cases where there were weakly positive UBT values (≥2.5 and <10.0), we were able to diagnose the correct status of the *H. pylori* infection after eradication in 105/106 (99.1%) of all patients (94.8–100%).

Table 2 Results of ^{13}C-urea breath test, *Helicobacter pylori* stool antigen test and histology in patients with discordant results

No.	Age/sex	UBT (per thousand)	HpSA	Histology
1	74/M	neg (0.0)	pos	pos
2	52/M	neg (0.6)	pos	neg
3	72/M	pos (2.6)	neg	neg
4	71/F	pos (2.7)	neg	neg
5	38/F	pos (4.4)	neg	neg
6	84/M	pos (4.7)	neg	neg
7	66/F	pos (6.0)	neg	neg
8	43/M	pos (11.1)	neg	pos
9	44/M	pos (13.2)	neg	pos

Underlined data indicates a result that is discordant with the histological examination. HpSA, *Helicobacter pylori* stool antigen test; neg, negative; pos, positive; UBT, ^{13}C-urea breath test.

Relationship between UBT, HPSA and histological result in cases with low UBT value

Among the 25 cases with a positive result of UBT, we detected a relatively low UBT value (≥2.5 and <10.0) in 10 cases. Five cases showed simultaneously positive reactions to the HpSA test, but the remaining five were judged as *H. pylori*-negative by the HpSA test, in which histological assessment also showed the clearance of *H. pylori* and the absence of active inflammation by histological examination (case 3–7 in Table 2).

DISCUSSION

The *H. pylori* stool antigen test is a new and valuable tool for the assessment of *H. pylori* infection without an invasive procedure[9] and is reported to have higher diagnostic accuracy.[10] Because it is a simple, economical and non-invasive examination, this method has found application in patients with pediatric disease,[11–15] acute gastrointestinal bleeding[16–18] and severe statuses such as liver cirrhosis.[19] It is likely that this tool will also be invaluable for large-scale studies such as the mass survey of gastric cancer.

Recent studies have shown that the HpSA test is a useful tool, not only for the primary diagnosis of *H. pylori* infection, but also for the evaluation of eradication therapy of *H. pylori*. Odaka *et al.*[20] and Tanaka *et al.*[21] have reported the usefulness of the HpSA test in the portion of the Japanese population undergoing eradication therapy, even in the short term.[20] Vaira *et al.* also showed that the HpSA test has an identical diagnostic value compared with the UBT and emphasized the clinical usefulness of the HpSA test for the monitoring of eradication therapy.[22] Ishihara *et al.* examined the correlation between the HpSA and UBT in patients 4 weeks after their eradication therapy and they showed a close relationship between the two methods.[23]

In contrast, some reports pointed out the lesser ability of the HpSA test to evaluate eradication therapy. Perri *et al.* showed the low sensitivity of the HpSA test compared with the UBT in patients who underwent eradication.[24] To the contrary, Bilardi *et al.* reported a high incidence of false positive results in the HpSA test compared with the UBT.[25] These data are still contradictory and the reason behind these discordant results is still under discussion.

One of the major problems in this field lies in the difference in standard examinations. When we regard UBT as the standard method, false negative cases with the HpSA test will naturally increase. In some reports, histological examination has been used as the standard, and this seems appropriate. However, until now, few reports discussed the histological changes based on the international rule of the updated Sydney system. Furthermore, using only the evaluation of the presence of bacteria, we were not able to exclude the possibility of false judgment even when using complicated methods such as immunohistochemical analysis. Focus must be placed on the improvement of active gastritis in the gastric mucosa, because this is the essential goal of eradication therapy.

In the present study, we showed the clinical usefulness of the HpSA test in the evaluation of eradication therapy in Japanese patients. The HpSA test showed a relatively higher prevalence of correct diagnoses compared with UBT. We detected discordant results of the UBT and the HpSA test in 8.6% of all cases (9/105), which is identical to the previous report.[24] However, we found more than half of the cases with discordant results were a result of the false positive result in the UBT, not the false negative in the HpSA test as previously reported. To improve the accuracy of the final decision on *H. pylori* infection following eradication therapy, we followed up for a sufficient period (more than 8 weeks) after the eradication. Previous reports had shown that more than 4 or 6 months are needed after eradication to exclude the false results. In addition, we examined the histological assessment with the use of the updated Sydney system.

False-positive results of the UBT test have already been reported in other studies. In the Japanese case, Kato *et al.* reported the justification of a new cut-off value (3.5 per thousand) for the UBT.[3] In the present study, when we set the cut-off for the UBT at 3.5 per thousand, the incidence of false positives decreased (from five to three cases) without an increase in false negatives (data not shown). However, we could not exclude the possibility of false positive cases completely. In practical use, a combination of the UBT and the HpSA test must be beneficial. When there is a low-grade positive value of the UBT (less than 10 per thousand), additional examination using the HpSA test is recommended. Although the gold standard test for *H. pylori* infection is still difficult to decide, at least in studies the authors of the present study have carried out, almost all patients received an accurate diagnosis that ultimately resulted in the improvement of their active gastritis. The UBT value of 10.0, which we described as the upper limit of low-grade positive, is not based on enough evidence, and further larger-scaled examination is needed to determine the proper cut-off value.

Another problem might be a result of the difference in the methods, such as the ELISA system used in the HpSA test and the cut-off value in these examinations. Recently, HpSA ELISA using monoclonal antibodies has been established, and reported similar results to that of the polyclonal antibody which was used in the present study.[26] The difference in the status of infection between nations might also be a problem. Particularly in Japanese populations, atrophic change is very common and the amount of *H. pylori* tends to be less than in Western populations.[6]

Taken together, HpSA is a very useful and non-invasive diagnostic tool for the evaluation of eradication therapy of *H. pylori* in Japanese patients. A combination of the use of the HpSA test and the UBT is very practical in the clinical evaluation of eradication therapy of *H. pylori*. Because simultaneous examination of UBT and HpSA test contains some practical and economic issues at present, this should be discussed further.

ACKNOWLEDGMENT

We thank Ms Yuko Ohtomo and Mr Yoshio Inoue (SRL Inc., Tokyo, Japan) for the technical assistance in examination of the *H. pylori* stool antigen test and in the production of database in this study.

REFERENCES

1 Asaka M, Kato M, Sugiyama T *et al.* Follow-up survey of a large-scale multicenter, double-blind study of triple therapy with lansoprazole, amoxicillin, and clarithromycin for eradication of *Helicobacter pylori* in Japanese peptic ulcer patients. *J. Gastroenterol.* 2003; **38**: 339–47.

2 Asaka M, Satoh K, Sugano K *et al.* Guidelines in the management of *Helicobacter pylori* infection in Japan. *Helicobacter* 2001; **6**: 177–86.

3 Kato C, Sugiyama T, Sato K *et al.* Appropriate cut-off value of ^{13}C-urea breath test after eradication of *Helicobacter pylori* infection in Japan. *J. Gastroenterol. Hepatol.* 2003; **18**: 1379–83.

4 Malfertheiner P, Megraud F, O'Morain C *et al.* European *Helicobacter Pylori* Study Group (EHPSG). Current concepts in the management of *Helicobacter pylori* infection – the Maastricht 2-2000 Consensus Report. *Aliment. Pharmacol. Ther.* 2002; **16**: 167–80.

5 Matsuda M, Noda Y, Takemori Y. Utility and limitations of a method for detecting *Helicobacter pylori*-specific antigens in the stool. *J. Gastroenterol.* 2003; **38**: 222–8.

6 Chen X, Haruma K, Kamada T *et al.* Factors that affect results of the ^{13}C urea breath test in Japanese patients. *Helicobacter* 2000; **5**: 98–103.

7 Dixon MF, Genta RM, Yardley JH *et al.* Classification and grading of gastritis. The updated Sydney System. International workshop on the Histopathology of gastritis. *Am. J. Surg. Path.* 1996; **20**: 1161–81.

8 Haruma K, Mihara M, Okamoto E *et al.* Eradication of *Helicobacter pylori* increases gastric acidity in patients with

atrophic gastritis of the corpus. Evaluation of 24-h pH monitoring. *Aliment. Pharmacol. Ther.* 1999; **13**: 155–62.
9 Trevisani L, Sartori S, Galvani F *et al.* Evaluation of a new enzyme immunoassay for detecting *Helicobacter pylori* in feces: a prospective pilot study. *Am. J. Gastroenterol.* 1999; **94**: 1830–33.
10 Vaira D, Malfertheiner P, Megraud F *et al.* Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. HpSA European Study Group. *Lancet* 1999; **354**: 30–33.
11 Okuda M, Nakazawa T, Booka M *et al.* Evaluation of a urine antibody test for *Helicobacter pylori* in Japanese children. *J. Pediatr.* 2004, **144**: 196–9.
12 Elitsur Y, Lawrence Z, Hill I. Stool antigen test for diagnosis of *Helicobacter pylori* infection in children with Symptomatic disease: a prospective study. *J. Pediatr. Gastroenterol. Nutr.* 2004; **39**: 64–7.
13 Sinha SK, Martin B, Gold BD *et al.* The incidence of *Helicobacter pylori* acquisition in children of a Canadian first nations community and the potential for parent-to-child transmission. *Helicobacter* 2004; **9**: 59–68.
14 Koletzko S, Konstantopoulos N, Bosman D *et al.* Evaluation of a novel monoclonal enzyme immunoassay for detection of *Helicobacter pylori* antigen in stool from children. *Gut* 2003; **52**: 804–6.
15 Kato S, Ozawa K, Okuda M *et al.* Accuracy of the stool antigen test for the diagnosis of childhood *Helicobacter pylori* infection: a multicenter Japanese study. *Am. J. Gastroenterol.* 2003; **98**: 296–300.
16 van Leerdam ME, van der Ende A, ten Kate FJ *et al.* Lack of accuracy of the noninvasive *Helicobacter pylori* stool antigen test in patients with gastroduodenal ulcer bleeding. *Am. J. Gastroenterol.* 2003; **98**: 798–801.
17 Peitz U, Leodolter A, Kahl S *et al.* Antigen stool test for assessment of *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. *Aliment. Pharmacol. Ther.* 2003; **17**: 1075–84.
18 Gisbert JP, Trapero M, Calvet X *et al.* Evaluation of three different tests for the detection of stool antigens to diagnose *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. *Aliment. Pharmacol. Ther.* 2004; **19**: 923–9.
19 Calvet X, Quesada M, Rosello M *et al.* Stool antigen for the diagnosis of *Helicobacter pylori* infection in cirrhosis: comparative usefulness of three different methods. *Aliment. Pharmacol. Ther.* 2003; **17**: 727–31.
20 Odaka T, Yamaguchi T, Koyama H *et al.* Evaluation of the *Helicobacter pylori* stool antigen test for monitoring eradication therapy. *Am. J. Gastroenterol.* 2002; **97**: 594–9.
21 Tanaka A, Watanabe K, Tokunaga K *et al.* Evaluation of *Helicobacter pylori* stool antigen test before and after eradication therapy. *J. Gastroenterol. Hepatol.* 2003; **18**: 732–8.
22 Vaira D, Vakil N, Menegatti M *et al.* The stool antigen test for detection of *Helicobacter pylori* after eradication therapy. *Ann. Intern. Med.* 2002; **136**: 280–87.
23 Ishihara S, Kaji T, Kawamura A *et al.* Diagnostic accuracy of a new non-invasive enzyme immunoassay for detecting *Helicobacter pylori* in stools after eradication therapy. *Aliment. Pharmacol. Ther.* 2000; **14**: 611–14.
24 Perri F, Manes G, Neri M *et al. Helicobacter pylori* antigen stool test and ^{13}C-urea breath test in patients after eradication treatments. *Am. J. Gastroenterol.* 2002; **97**: 2756–62.
25 Bilardi C, Biagini R, Dulbecco P *et al.* Stool antigen assay (HpSA) is less reliable than urea breath test for post-treatment diagnosis of *Helicobacter pylori* infection. *Aliment. Pharmacol. Ther.* 2002; **16**: 1733–8.
26 Leodolter A, Peitz U, Ebert MP *et al.* Comparison of two enzyme immunoassays for the assessment of *Helicobacter pylori* status in stool specimens after eradication therapy. *Am. J. Gastroenterol.* 2002; **97**: 1682–6.