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Helicobacter pylori infection in the remant stomach after gastrectomy for gastric cancer or peptic ulcer: Preliminary results

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Helicobacter pylori infection contributes to many gastric diseases like peptic ulcers and gastric cancer. However, few reports mentioned in *H. pylori* infection after gastrectomy. In this study, we examined *H. pylori* infection in the remnant stomach by the PCR method with gastric juices and *H. pylori* IgG antibody (ELISA).

From October 1991 to August 1996, endoscopic examinations were carried out in the First Department of Surgery (Nippon Medical School) and sera were collected from 207 gastric cancer or peptic ulcer patients. *H. pylori* DNA in the gastric juices was amplified by PCR and detected by agarose gel electrophoresis and Southern blot hybridization. *H. pylori* DNA in the gastric juices was examined in 66 patients (mean 62 years, M/F = 50/16) after gastrectomy for gastric cancer (50 patients) or peptic ulcer (16 patients). Serum IgG antibody against *H. pylori* (ELISA) was examined in 111 patients (mean 61.6 years, M/F = 76/35) after gastrectomy for gastric cancer (93 patients) or peptic ulcers (18 patients). *H. pylori* infection was found in 48/66 (72.7%), and 89/111 (80.2%) patients by PCR and ELISA, respectively.

No significant differences in *H. pylori* positivity with either PCR or ELISA were found according to age group, sex, time after operation, or disease (gastric cancer or peptic ulcers). Only the type of anastomosis showed statistical significance by the gastric juice-PCR method (P = 0.014). Billroth II anastomosis decreased the rate of *H. pylori* infection in 7/15 (46.7%), and Billroth I anastomosis in 37/45 (82.2%).

It is concluded that there are no factors affected the positivity of *H. pylori* in gastric remnant except the type of anastomosis, where a lower incidence of infection was found in Billroth II anastomosis than in Billroth I. This might reflect the role of bile reflux, which is more common in Billroth II than in Billroth I, since bile reflux disturbs *H. pylori*'s colonization in gastric mucosa.

Index : *H. pylori*, gastric remnant

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Chronic gastritis in the gastric remnant after distal gastrectomy for gastric cancer or peptic ulcers is important because cancer in the residual stomach are detected occasionally. It is well known that reflux of bile acid is the major cause of gastritis (alkaline reflux gastritis, ARG). Advances in the understanding of *H. pylori* infection have revealed this organism can cause chronic gastritis[1-4], recurrence of peptic ulcers[5] and can probably induce gastric cancer[4,6-11]. However, in the past ten years, there have been a few studies about the contribution of *H. pylori* infection in the gastric remnant.

Among previous data, some showed that *H. pylori* seem to be found with low incidence in the residual stomach[12-18]. The data from Japan also showed the same tendency[19-21]. Bile reflux was discussed as the possible cause of *H. pylori* eradication in such patients[12-15,17,19-25]. But this theory is still being debated by other investigators[16,26-27].

Differences in surgical procedures are reported to be one of the factors in the positivity of *H. pylori* postoperatively. Especially, very few published reports have studied the difference in infection rates between Billroth I and II[13,20,22]. We conducted a study in gastrectomized patients, comparing various factors that might affect the positivity of *H. pylori*. These factors, including age, sex, type of disease, type of operation and time after operation were compared by means of the PCR method of detection of *H. pylori* DNA in gastric juice as well as serum IgG antibody against *H. pylori*.

Patients and methods

Patients

Clinical study at the First Department of Surgery, Nippon Medical school, Tokyo, Japan. From October 1991 to August 1996, 242 gastrectomized patients was investigated by endoscopic examination (mean age 59.8 years, range 25-85 years, M/F = 169/73). Two hundred and seven patients were diagnosed with peptic ulcers or gastric cancer (duodenal ulcers 11 cases, gastric ulcers 26 cases, gastroduodenal ulcer 1 case, and gastric cancer 169 cases). These patients (mean age 60.6 years, range 30-85 years, M/F = 142/65), were examined by means of PCR of gastric juices (66 patients, mean age 62 years, range 32-85 years, M/F = 50/16), and by serum IgG antibody against *H. pylori* (111 patients, mean age 61.6 years, range 30-85 years, M/F = 76/35).

Sampling of gastric juice

About 5 ml of gastric juices was collected with a sterile suction tube through the biopsy suction channel of a gastrofiberscope. Sampling details were reported previously (Matsukura et al[28]).

DNA extraction

Approximately 1 ml of gastric juices was neutralized with 1 N NaOH and centrifuged at 15000 rpm for 10 minutes. The pellet was resuspended in 0.5 ml tissue extraction buffer for 2 hours at 56°C. The DNA was extracted with phenol-chloroform, and precipitated with ethanol, and the pellet was dried and dissolved in 50 ml TE buffer (10 mM Tris hydrochloride, and 1 mM EDTA).

PCR primers and amplification

The following primer pair, which was homologous to a portion of the 1.9-kb fragment of chromosomal DNA of *H. pylori*, was used as described by Valentine et al[29]: CAM-2 primer (sense), 5′-(CATCTTGTTAGA GGGATTGG)-3′ and CAM-4 primer (antisense), 5′-(TAACAAACCGATAATGGCGC)-3′. PCR primers and a probe were chemically synthesized on a Cyclone Plus DNA synthesizer (Millitore Co., Bedford, Mass.). A heating block (Gene Amp PCR system 9600-R; Nippon Roche K.K., Tokyo, Japan) was used for thermocycling. The total volume (50 μl) of each reaction sample contained 200 mM each of four deoxynucleotides (Takara Shuzo Co., Ltd., Kyoto, Japan), 0.3 μM of each primer in a standard PCR incubation buffer, 1.25 U AmpliTaq polymerase (Nippon Roche K.K.), and 10 ml DNA. Cycling conditions were: denaturation at 94°C for 0.5 minute, annealing at 55°C for 1.0 minute, and extension at 72°C for 1.0 minute. A total of 50 cycles were performed.

Agarose gel electrophoresis and Southern blot hybridization

The amplified products were analyzed by electrophoresis on 3.0% agarose gels and stained with ethidium bromide to detect DNA fragments of 203 base pairs. The gels were photographed under ultraviolet (UV) illumination. An internal DNA probe, CAM-3,5′-(CGCTCTTTAGTTTTGGAGCG)-3′ end-labeled with [^{32}P]ATP (Du Pont Co., Wilmington, Del.) was used. This was hybridized to the PCR products on a nylon membrane (Biodyne B: Pall Ultrafine Filtration Co. Ltd., New York, N.Y.) at 55 °C for 2 hours. The *H. pylori*-specific oligonucleotide probe was hybridized to a specific sized band (203 base pairs). The filters were washed for 5 minutes at room temperature in 5 x standard saline citrate (SSC) and 0.1 x SSC containing 0.1% Sodium dodecyl sulfate (SDS) (twice) and finally washed with 5 x SSC-0.1% SDC (twice) for 10 minutes at 50 °C.

Sensitivity and specificity of the PCR assay

Sensitivity, specificity, and accuracy of PCR assay were 87%, 79%, and 84% by bacterial culture as standard, and 76%, 90%, and 83% by ^{13}C-UBT as standard in non-surgical stomach. Sensitivity, specificity, and accuracy of serum IgG ELISA assay were 100%, 13%, and 55% by bacterial culture as standard, and 99%, 14%, and 53% by ^{13}C-UBT as standard in non-surgical stomach.

Serum IgG antibody against H. pylori

Blood samples were taken and stored at -80 °C until analysis. Serum IgG against *H. pylori* was assayed by ELISA (AMRAD Operation Pty Ltd., Australia) and the absorbance at 450 nm was measured. The results were expressed quantitatively. Less than 30 U/ml was regarded as negative.

All assays were carried out at Mitsubishi-Kagaku Bio-Clinical Laboratory (Tokyo, Japan) as a cooperative study.

Statistical analysis

Data were analyzed by the x^2 test and Student-*t* test. Values of $p < 0.05$ were taken to indicate significance.

Results

H. pylori prevalence

All of the 242 patients were analyzed from the point of view of various factors that may have affected positivity of *H. pylori* in gastric remnant. Two hundred and seven patients were diagnosed with peptic ulcers or gastric cancer (duodenal ulcers 11 cases, gastric ulcers 26 cases, gastroduodenal ulcer 1 case, and gastric cancer 169 cases). These patients (mean age 60.6 years, range 30-85 years, M/F = 142/65), were examined by means of PCR of gastric juices (66 patients, mean age 62 years, range 32-85 years, M/F = 50/16), and by serum IgG antibody against *H. pylori* (111 patients, mean age 61.6 years, range 30-85 years, M/F = 76/35). *H. pylori* infection was found in 48/66 (72.7%), and 89/111 (80.2%) patients by PCR and ELISA, respectively.

The factors analyzed were:

Age. Age distributions are shown in Table 1. Mean age was 59.8 years. There are no statistical differences among patients investigated by gastric juice-PCR (n = 68, mean age 61.6 years) and serum IgG (ELISA) (n = 125, mean age 61.3 years). Positivity of *H. pylori* according to age distribution is shown in Table 2. There are no statistical differences in *H. pylori* infection among the age groups of patients, neither PCR nor ELISA.

Sex. The male/female ratio is shown in Table 1. We examined 169 male patients, and 73 female patients. Table 2 shows that this factor was not statistically significant regarding *H. pylori* positivity.

Type of disease. The diseases operated on are shown in Table 1. To compare between gastric cancer and peptic ulcers, 207 cases were studied: 169 cases of gastric cancer and 38 cases of peptic ulcers (duodenal ulcers: 11 cases, gastric ulcers: 26 cases, and gastroduodenal ulcer: 1 case). The comparative positivity

Table 1 Patients characteristics who underwent distal gastrectomy for gastric cancer or peptic ulcer and endoscopy, PCR examination for *H. pylori* in gastric juice and serum IgG antibody against *H. pylori* (ELISA) were performed after operation

Variables	Endoscopy (n)	PCR (n)	ELISA (n)
No. of patients	242	68	125
Age:			
mean	59.8 yrs	61.6 yrs	61.3 yrs
≤49	39	9	21
50-59	65	19	30
60-69	79	23	43
≥70	59	17	31
Sex:			
Male	169	52	87
Female	73	16	38
Type of disease: *			
GCA	169	50	93
PU	38	16	18
Others+no data**	35	2	14
Anastomosis:**			
B I	137	45	76
B II	48	15	26
Others+no data**	57	8	23
Time after operation:			
mean (yrs)	5	5.5	5
≤1	42	12	21
1-5	98	33	51
≥5-10	36	9	22
≥10	26	10	12
no data	40	4	19

* *GCA, gastric cancer; PU, peptic ulcer; others, malignant lymphoma*

** *no data, Patients with no data available were excluded from further analysis.*

*** *B I, Billroth I; B II, Billroth II; others, Roux-en-Y*

Table 2 Positivity or negativity of *H. pylori* by PCR or ELISA compared with demographics, type of disease, anastomosis and time after operation

Variables	*H. pylori* assay*					
	PCR			ELISA		
	+	-	*p*Value	+	-	*p*Value
No. of patients	50	18		97	28	
Sex :						
Male	36	16		66	21	
Female	14	2	ns	31	7	ns
Age (yrs) distribution :						
≤49	4	5		17	4	
50-59	18	1		28	2	
60-69	14	9		30	13	
≥ 70	14	3	ns	22	9	ns
Type of disease :						
GCA	37	13		73	20	
PU	11	5	ns	16	2	ns
Anastomosis :						
B I	37	8		63	13	
B II	7	8	$P = 0.014$	20	6	ns
Time after operation (yrs) :						
≤ 1	5	7		20	1	
1-5	25	8		40	11	
≥ 5-10	7	2		16	6	
≥ 10	9	1	ns	10	2	ns

** + = positive; - = negative; ns= not significant*

of *H. pylori* infection due to gastric cancer and peptic ulcers detected by PCR or ELISA is not statistically significant, as is shown in Table 2.

Type of operation. In Japan, Billroth I operation is the standard procedure for gastric cancer or peptic ulcer cases. The number of patients undergoing Billroth I or II operations are shown in Table 1. There were a few patients who underwent Roux-en-Y anastomosis, but they are excluded from this study. The positivity of *H. pylori* was significantly lower in patients undergoing Billroth II than Billroth I, as detected by gastric juice-PCR (*p* Value = 0.014). The results are shown in Table 2.

Time after operation. Times after operation are shown in Table 1. The mean time of follow-up was 5 years. In patients investigated by PCR or ELISA, the mean follow-up periods were 5.5 years and 5 years, respectively. Positivity of *H. pylori* infection due to this factors was not statistically significant, as is shown in Table 2.

Discussion

Postoperative alkaline reflux gastritis is now being studied worldwide in the field of clinical gastroenterology. The reason for focusing on the postoperative residual stomach is the recurrence of stomal ulcers or cancer. Even more, the new occurrence of gastric cancer, in areas distant from the stomal site in patients who had been operated on for a period of time had been mentioned[31]. But, since the discovery of *H. pylori* in early 1980s, close correlation between *H. pylori* infection and gastric cancer incidence was reported, and IARC/WHO defined *H. pylori* infection as a definite carcinogenesis[32]. However, a few investigators have become interested in this organism with regard to its role in the induction of gastritis in the residual stomach.

Published data show that the infection rate of gastric remnant was significantly decreased, compared to normal population in each countries[12-18]. This may be because of the antrum of the stomach in which *H. pylori* favorably colonized was removed by operation and possibly the bile reflux process can eradicate *H. pylori*[12-15,17,19-25], because bile suppress *H. pylori* growth *in vitro*[33]. But the matter is still controversial. In the past ten years, there have been few reports on *H. pylori*-induced residual gastritis after gastrectomy in comparison with alkaline reflux gastritis. Several papers seem to agree on the *H. pylori* eradication potential of bile reflux[12-15,17,19-25]. But different clinical data also reported[16,26,27]. The problem would seem to lie in the quantity of bile reflux and the reliability of the method of bile detection.

Recent studies from German[17] and Japan[19-21] have shown that, according to the method used to detect *H. pylori* in gastric remnant. The incidence is 20.8-56%. Other one investigator[34] compared a chromatographic methods for detection of *H. pylori* in gastric juice with serum IgG and found the positivity was 62.4-69.9%.

In our study, we used the PCR method for detection of *H. pylori* DNA in gastric juice as well as serum IgG antibody against *H. pylori* because these two methods represent the whole remnant stomach study. Particularly, the sensitivity and specificity of PCR detection of *H. pylori* in gastric juice is high and reliable[28]. In this study, using this sensitive PCR and ELISA method, the positivity of this microorganism are 72.7% and 80.2% by PCR and serum *H. pylori* IgG antibody, respectively.

Various factors such as age, sex, type of disease, and time after operation did not effect the positivity of *H. pylori* in residual stomach. The type of anastomosis, however, was significant in the Billroth II operation produced a statistically significant lower positivity.

Our results in the PCR method in gastric juices showed that *H.pylori* positivity was significantly higher in Billroth I operation (82.2%, 37/45) than in Billroth II operation (46.7%,7/15). This study did not investigate alkaline reflux gastritis directly, but several data indicated bile refluxs facility to eradicate *H. pylori* in gastric remnant. It is plausible that bile acid eradicate *H. pylori* in Billroth II anastomosis. In previous literature[20,22], there was no significant difference in *H. pylori* infection in residual stomach between Billroth I and II operation (B I = 72%, B II = 65% detected by histology[22], and B I = 49% , B II = 37% detected by culture[20], although B II showed

lower positivity than B I). The study found Billroth II operation had a significantly lower infection rate, than in Roux-en-Y operation was shown by few investigators[23]. They suggested that reflux gastritis are distinct microscopic entities and that bile reflux may play a role in the eradication of *H. pylori* after gastrectomy. One report[14] clearly identified a higher incidence of infection after Roux-en-Y procedure (preoperative infection rate = 54%, postoperative infection rate = 92%, detected by histology). We have the same opinion about the eradicational effect of reflux bile in gastric remnant after gastrectomy with Billroth II anastomosis.

There is one suggestion that previous *H. pylori*-induces gastritis in a preoperative case can change to alkaline reflux gastritis after gastrectomy [12-14]. We agree to this axis but not at all. From our data, we suggest that this axis has a good correspondence to patients who undergo Billroth II operation because of the high incidence of alkaline reflux gastritis in Billroth II operation[35] and eradicational effect of bile acid on *H. pylori*. But in Billroth I operation, this axis seems to be elucidated because our data show a high incidence of residual gastric *H. pylori* infection, probably corresponding to a low incidence of alkaline reflux gastritis. Therefore, gastric remnant with Billroth I anastomosis, *H. pylori* infection may play important role for gastritis in remnant stomach. Further study is needed to clarify in this point.

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