

Genotypes of *Helicobacter pylori* in patients with peptic ulcer bleeding

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Abstract

AIM: *Helicobacter pylori* causes chronic gastritis, peptic ulcer, gastric cancer and MALT-lymphoma. Different genotypes of *Helicobacter pylori* are confirmed from diverse geographic areas. Its association with bleeding peptic ulcer remains controversial. The aim of this study was to investigate the *Helicobacter pylori vacA* alleles, *cagA* and *iceA* in patients with bleeding peptic ulcer.

METHODS: We enrolled patients with bleeding, non-bleeding peptic ulcers and chronic gastritis. Biopsy specimens were obtained from the antrum of the stomach for rapid urease test, bacterial culture and PCR assay. DNA extraction and polymerase chain reaction were used to detect the presence or absence of *cagA* and to assess the polymorphism of *vacA* and *iceA*.

RESULTS: A total of 168 patients (60.4%) (25 patients with chronic gastritis, 26 patients with bleeding gastric ulcer, 51 patients with non-bleeding gastric ulcer, 26 patients with bleeding duodenal ulcer, and 40 patients with non-bleeding duodenal ulcer) were found to have positive PCR results between January 2001 and December 2002. Concerning genotypes, we found *cagA* (139/278, 50%), *vacA* s1a (127/278, 45.7%), and *iceA* A1 (125/278, 45%) predominated in all studied patients. In patients with bleeding peptic ulcers, *vacA* s1a and m1T were fewer than those in patients with non-bleeding peptic ulcers (37/106 vs 69/135, $P=0.017$, and 4/106 vs 21/135, $P=0.002$).

CONCLUSION: In patients with peptic ulcers, *H pylori vacA* s1a and m1T prevent bleeding complication.

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INTRODUCTION

Helicobacter pylori (*H pylori*) infection has been closely linked

to chronic gastritis, peptic ulcer, gastric cancer and MALT-lymphoma^[1]. It is one of the most common bacterial infections of humans^[2]. It remains to be answered why only a minority of *H pylori* carriers develop peptic ulcer disease. Host factors, *H pylori* strain variability, environmental factors, and NSAID play a role in the pathogenesis of peptic ulcer disease^[3-5]. The clinical outcome of *H pylori* infection is supposed to be linked to certain strains, e.g. vacuolating cytotoxin (*vacA*) and the cytotoxin-associated gene (*cagA*)^[6,7].

In patients with peptic ulcer disease, only a minority of them present with peptic ulcer hemorrhage. The incidence of peptic ulcer hemorrhage in patients with pre-existing peptic ulcer disease is less than 1% per year^[8]. Whether *H pylori* increases the risk of ulcer bleeding is controversial. Wu *et al* confirmed that *H pylori* increased the risk of peptic ulcer bleeding^[9]. Cullen *et al* had a similar finding (OR 2.8)^[10]. In addition, eradication of *H pylori* infection could decrease the chance of peptic ulcer bleeding^[11,12]. The above evidences strongly support the link of *H pylori* to peptic ulcer bleeding. However, the prevalence of *H pylori* has been found to be lower in bleeding ulcer patients than in non-bleeders^[13]. The most likely explanation is the use of NSAIDs in the absence of *H pylori* infection in these patients. Another reason may be false negative results in these patients^[14,15]. If we excluded the usage of NSAIDs in patients with duodenal ulcer bleeding, the prevalence of *H pylori* infection was 97%^[16].

Controversy exists concerning relationship of genotypes of *H pylori* with peptic ulcer bleeding. So far, there are only few reports concerning this topic^[17,18]. Although *H pylori* infection is very common, geographic distribution of different subtypes exists^[19,20]. Therefore, it is interesting to investigate the genotypes in patients with peptic ulcer bleeding. The aim of this study was to determine the genotypes of *H pylori* in bleeding ulcer patients in Taiwan.

MATERIALS AND METHODS

Between January 2001 and December 2002, patients with non-bleeding peptic ulcers (gastric ulcer or duodenal ulcer, at least 5 mm in diameter), bleeding peptic ulcers (spurting or oozing hemorrhage, non-bleeding visible vessel, blood clots or pigmented spots at the ulcer base) or chronic gastritis were invited to enter the study. There was no past history of upper gastrointestinal bleeding (hematemesis or melena) in patients with non-bleeding peptic ulcer in this study. Patients with pregnancy, bleeding tendency (platelet count less than 50 000/mm³, prothrombin time less than 30%, or taking anti-coagulants), gastric malignancy, age under 10, or over 90 years, anti-*H pylori* therapy 4 weeks prior to enrollment, and inability to cooperate were excluded from the study. The study was approved by the Clinical Research Committee of the Veterans General Hospital, Taipei.

Endoscopic examination and biopsy were performed after informed consent was obtained. We took three specimens from the antrum, one for rapid urease test, another for bacterial culture and the third for DNA extraction and PCR assay. Lysates of biopsied gastric mucosa were used for PCR assay. DNA of gastric biopsy specimens was extracted according to the method described by Boom^[21]. Briefly, biopsy specimens

were homogenized in guanidinium isothiocyanate, using a sterile micropestle. DNA was extracted, washed and eluted in 100 µl of 10 mM Tris-HCL (pH 8.3). Two µl of the eluted DNA was used for each PCR reaction.

The oligonucleotide primers for PCR amplification of specific segments are shown in Table 1^[5,22-25]. For *vacA* evaluation, the PCR program comprised 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, extension at 72°C for 1 min, and one final extension at 72°C for 10 min. For *cagA*, amplification was performed with 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, extension at 72°C for 1 min, and one final extension at 72°C for 5 min. For *iceA* amplification, amplifications were performed with 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 45 s, extension at 72°C for 45 s and one final extension at 72°C for 10 min.

The association between *H pylori* genotypes and clinical diseases was determined using χ^2 test and Yates' correction or Fisher's exact test when appropriate. A *P* value less than 0.05 was considered statistically significant.

RESULTS

A total of 278 patients with bleeding or non-bleeding peptic ulcers and chronic gastritis (200 males and 78 females, mean age: 62.1 years, 95% CI: 60.1-64.1 years) fulfilling the admission criteria, were included in this study. A total of 168 patients (60.4%) were found to have a positive urease test. A total of 168 patients (60.4%) (25 patients with chronic gastritis, 26 patients with bleeding gastric ulcer, 51 patients with non-bleeding gastric ulcer, 26 patients with bleeding duodenal ulcer, and 40 patients with non-bleeding duodenal ulcer) were found

Table 1 Oligonucleotide primers used for *cagA*, *vacA* and *iceA* genotyping

Region detected	Primer designation	Primer sequence	Size of PCR product (bp)	References
s1 and s2	VA1-F	5' ATGGAAATACAACAAACACACC3'	259	21
	VA1-R	5' CTGCTTGAATGCGCCAAACTTTATC3'	286	
s1a	SS1-F	5' GTCAGCATCACACCGCAAC3'	190	9
s1b	SS3-F	5' AGCGCCATACCGCAAGAG3'	187	9
s1c	S1C-F	5' CTYGCTTTAGTRGGGYTA-3'	213	17
m1	VA3-F	5' GGTCAAAATGCGGTCATGG3'	290	9
	VA3-R	5' CCATTGGTACCTGTAGAAAC3'		
m1T	m1T-F	5' GGTCAAAATGCGGTCATGG3'	290	21
	m1T-R	5' CTCTTAGTGCCTAAAGAAACA3'		
m2	VA4-F	5' GGAGCCCCAGGAAACATTG3'	352	9
	VA4-R	5' CATAACTAGCGCCTTGAC3'		
iceA1	iceA1F	5' GTGTTTTTAACCAAAGTATC3'	247	16
	iceA1R	5' CTATAGCCASTYTCTTTGCA3'		
iceA2	iceA2F	5' GTTGGGTATATCACAATTTAT3'	229	16
	iceA2R	5' TTRCCCTATTTTCTAGTAGGT3'		
lcagA	lcagAD008	5' ATAATGCTAAATTAGACAACCTTGAGCGA3'	297	5
	lcagAR008	5' TTAGAATAATCAACAAACATCACGCCAT3'		

Table 2 Genotypes of *Helicobacter pylori* in patients with chronic gastritis, non-bleeding duodenal ulcers (DU), bleeding DU, non-bleeding gastric ulcers (GU) and bleeding GU

Diagnosis	No. of patients	No. of positive PCR	s1a	s1b	s1c	s2	m1	m1T	m2	<i>cagA</i>	<i>iceA1</i>	<i>iceA2</i>
Chronic gastritis	37	25	21(57)	0(0)	14(38)	0(0)	0(0)	8(22)	12(32)	22(59)	22(59)	2(5)
Non-bleeding DU	53	40	30(57)	4(8)	22(42)	0(0)	0(0)	11(21)	15(28)	29(55)	30(57)	3(6)
Bleeding DU	48	26	17(35)	4(8)	15(31)	2(4)	2(4)	3(6)	12(25)	25(52)	19(40)	7(15)
Non-bleeding GU	82	51	39(48)	0(0)	29(35)	0(0)	0(0)	10(12)	30(37)	41(50)	34(41)	13(16)
Bleeding GU	58	26	20(34)	1(2)	13(22)	0(0)	0(0)	1(2)	15(26)	22(38)	20(34)	4(7)

$P > 0.05$ versus variables between non-bleeding DU and bleeding DU, between non-bleeding GU and bleeding GU.

Table 3 Genotypes of *Helicobacter pylori* in patients with non-bleeding peptic ulcers and bleeding peptic ulcers

Diagnosis	No. of patients	No. of positive PCR	s1a	s1b	s1c	s2	m1	m1T	m2	<i>cagA</i>	<i>iceA1</i>	<i>iceA2</i>
Non-bleeding PU	135	91	69 ^a (51)	4(3)	51(38)	0(0)	0(0)	21 ^b (16)	45(33)	70(52)	64(47)	16(12)
Bleeding PU	106	52	37(35)	5(5)	28(26)	2(2)	2(2)	4(4)	27(25)	47(44)	39(37)	11(10)

^a $P = 0.017$ between non-bleeding peptic ulcers and bleeding peptic ulcers. ^b $P = 0.002$ between non-bleeding peptic ulcers and bleeding peptic ulcers.

to have positive PCR results. The ages of patients with bleeding gastric ulcer (mean: 67.8 yr, 95% CI: 62.8-72.8), non-bleeding gastric ulcer (mean: 63.5 yrs, 95% CI: 59.8-67.2), bleeding duodenal ulcer (mean: 65.5 yrs, 95% CI: 59.2-71.8) were greater than those of patients with non-bleeding duodenal ulcer (mean: 54.6 yrs, 95% CI: 49.5-59.7, $P < 0.01$) and chronic gastritis (mean: 51.2 yrs, 95% CI: 42.8-59.6, $P < 0.01$).

In patients with bleeding gastric ulcer, there were blood clots inside the stomach in 8 patients, coffee grounds in 8 patients, and clear fluid in 10 patients. In patients with bleeding duodenal ulcers, there were blood clots inside the stomach in 8 patients, coffee grounds in two patients and clear fluid in 16 patients.

In patients with bleeding peptic ulcers, 52 (49.1%) were found to have positive PCR for *H pylori*. It was lower than that in those with non-bleeding peptic ulcers (91/135, 67.4%, $P = 0.006$) and chronic gastritis (25/37, 67.6%, $P = 0.008$).

The genotypes in patients with chronic gastritis, duodenal ulcers and gastric ulcers are described in Table 2. There was no statistical difference among variables in different groups.

Concerning genotypes, we found *cagA* (139/278, 50%), *vacA* s1a (127/278, 45.7%), and *iceA* A1 (125/278, 45%) predominated in all studied patients. In patients with bleeding peptic ulcers, *vacA* s1a and m1T were fewer than those in patients with non-bleeding peptic ulcers (37/106 vs 69/135, $P = 0.017$, and 4/106 vs 21/135, $P = 0.002$, Table 3).

DISCUSSION

H pylori is a world-wide infective agent ranging from 25% in developed countries to more than 80% in the developing world^[26,27]. However, not all individuals infected with *H pylori* develop gastric illness and this may be related to various factors such as environmental factors, host genetic factors, and bacterial virulent ability^[28,29].

This study determined using PCR the *cagA*, *vacA* and *iceA* status of 278 patients presenting for endoscopy by examining biopsy specimens from the antrum of each patient. Of them, 168 patients (60.4%) were found to have positive PCR. In this study, predominance of *vacA* s1a was found in patients with bleeding and non-bleeding peptic ulcer and chronic gastritis. Our finding was similar to those reported by other authors^[25,30,31]. In Hong Kong and Korea, a low incidence of *vacA* s1a subtype was found^[32,33].

The previous Taiwan reports gave no data concerning *vacA* s1c^[25,30]. *VacA* s1c was frequently found (93/278, 33.5%) in this study. In patients with bleeding peptic ulcers, *vacA* s1c was less than that in patients with non-bleeding peptic ulcers (26% vs 38%), but it did not reach statistical significance. The incidence of *vacA* s1c in this study was similar to the reports of Hong Kong^[32], Korea^[33], and Japan^[34], but different from those in Western world^[19,20]. In contrast, *vacA* s1b and s2 were rare. Our findings were compatible with that in mainland China^[31].

Concerning the m-region of *vacA*, m1 strains predominated in most Western reports^[19,20,27]. However, there were only 2% m1 subtypes in patients with bleeding peptic ulcers and none in patients with non-bleeding peptic ulcers in this study. We used a modified primer (m1T)^[25] and some peptic ulcer patients (bleeding: 3.8%, non-bleeding: 15.6%) with *H pylori* infection contained this genotype. m2 strains predominated (33% in patients with non-bleeding peptic ulcers, 25% in bleeding peptic ulcers) in this study. Our finding was consistent with reports from Taiwan^[25,30], Hong Kong^[32], and mainland China^[31]. In contrast, Japan and Korea had a much lower incidence of m2 strains^[24,33]. This indicates a great variation in the *vacA* region in Taiwan, particularly in the mid-region locus. *H pylori* may have a different geographic evolution in Taiwan

even compared with other East Asian countries.

IceA1 has been suggested to be related to peptic ulcer disease^[23,35]. But, this finding was doubted by other authors and us^[24,32,33]. In this study, we found *iceA1* was the predominant subtype and showed no difference between patients with bleeding and non-bleeding peptic ulcers. *IceA1* is the predominant subtype of *ice* in the East Asia, while *iceA2* is the predominant subtype in the USA and Columbia^[24].

Certain genotypes (e.g. *cagA*, *vacA* s1a) have been closely related to severe clinical outcomes and response to anti-*H pylori* therapy^[36-38]. However, these findings are not supported by other studies^[24,25,29-32,34]. The association between *H pylori* infection and peptic ulcer bleeding is less clear, but a strong argument for the etiological role is the fact that eradication of *H pylori* decreased recurrence of bleeding^[39]. Stack *et al* recently found that *cagA* positive *H pylori* was associated with an increased risk of ulcer bleeding^[17]. However, Illies *et al* found that presence of *cagA* antibody was similar both in patients with bleeding and in non-bleeding controls^[18]. In this study, there was no difference of *cagA* between patients with non-bleeding and those with bleeding peptic ulcers. However, there were fewer *vacA* s1a and m1T in patients with bleeding peptic ulcers than in patients with non-bleeding peptic ulcers.

In conclusion, in patients with bleeding peptic ulcers, *H pylori vacA* s1a and m1T are less than those in patients with non-bleeding peptic ulcers.

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