Helicobacter pylori virulence factors and their role in peptic ulcer diseases in Turkey

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Abstract

Background and study aims : **The role of virulence factors present in *Helicobacter pylori (H. pylori)* strains and the characterization of such factors being predictive of specific disease is still not clear. In this study, the *cagA*, *vacA* alleles and the recently characterized *vacA* i-region and *dupA* and their association with the severity of the disease was determined.**

Patients and methods : **Antral biopsies from 91 patients with peptic ulcer (PU) (n = 41), gastritis (n = 48) and gastric cancer (GC) (n = 2) were analyzed for the presence of *H. pylori* by the CLO-test® and PCR. A 79/91 (86%) patients were positive for *H. pylori* by either PCR or by both PCR and CLO-test®. PCR-based typing of *H. pylori* isolates was performed on DNA extracted directly from biopsy samples.**

Results : **The *cagA*+ strains were found more likely to be associated with *vacA* s1 than s2. The *vacA* i1 allele detected in 16/23 (70%) of samples had significant association with duodenal ulcers than those 16/37 (44%) of gastritis ($P < 0.04$). No significant association was found between *dupA* and duodenal ulcer. This study provided more evidence that the *vacA* i1 allele is one of the virulence factors of *H. pylori* that had significant association with severe outcome.** (Acta gastroenterol. belg., **2010**, 73, **235-238**).

Key words : H. pylori, cagA, vacA, dupA, vacA i-region.

Introduction

Helicobacter pylori (*H. pylori*) infection in most individuals is asymptomatic while many live with chronic gastritis ; some develop peptic ulcer diseases (PUD) and still few may progress to gastric cancer (1-3). This was attributed to the presence of virulence factors by certain *H. pylori* strains (4,5). The characterization of such factors predictive of association with disease is still unclear. Of the several *H. pylori* virulence factors that may play a role in pathogenesis, the *cagA* gene (present in around 68% of the strains) and the *vacA* gene (present in all strains) are found to be associated with more severe diseases (4,6). The *vacA* gene exists in 2 polymorphic forms : the s (signal) and m (middle) regions, and recently a novel determinant, the i (intermediate) region existing as i1 and i2 allelic variants (7). It was reported that the *vacA* i-region is an important determinant of toxicity, being the best independent marker of *vacA*-associated pathogenicity. The duodenal ulcer promoting gene A (*dupA*) is another recently described virulence factor which encompasses the *jhp0917* and *jhp0918* genes of the plasticity region, that was also found to have a significant association with duodenal ulcers (DU) (8). Previous reports from Turkey showed that around 70% of *H. pylori* strains typed positive for *cagA*. In addition, a significant correlation was found between *cagA*+ *vacA*s1 genotype and PUD (9,10). The aim of this study was to investigate the association between the above mentioned virulence factors, in particular the newly described *vacA* i region and *dupA*, and the clinical outcome in Turkish patients.

Materials and methods

Collection of biopsy samples

Three antral biopsies from 91 Turkish patients (50 females) of 16-79 years of age (average 40) with peptic ulcer (n = 41), gastritis (n = 48) and gastric cancer (GC) (n = 2) consecutively attending the Istanbul Teaching Hospital from January 2008 to June 2009 were analyzed. Patients on antibiotics, NSAIDs or PPIs 4 weeks before endoscopy were excluded. Approval of the ethical committee and patient consent was obtained. One biopsy was used for CLO test to detect the presence of *H. pylori* and the other two for genotyping of *H. pylori* directly from biopsies by PCR.

DNA extraction

The QIAamp DNA Mini Kit (Qiagen Co., Hilden, Germany) was used for DNA extraction.

PCR

The methods described previously were used (Table 1). Primer concentrations and annealing temperature adjustment for each method was applied. In general, an initial denaturation cycle for 5 minutes at 95°C, followed by 35 cycles at 95°C for 20 seconds, 55°C for 20 seconds and 72°C for 40 seconds, and a final cycle at 72°C for 7 minutes. The amplified products were visualized by agarose gel electrophoresis. The primers used for the amplification of the genes were listed in Table 1.

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Table 1. — **The primers used for the amplification of the *H. pylori* genes**

Gene	Primer	5' - 3' sequence	Reference
cagA	cag5c-F cag3c-R	GTTGATAACGCTGTCGCTTC GGGTTGTATGATATTTTCCATAA	(10)
dupA	*Dup*AF1202 *Dup*A918R	TAAAATCACAAGGGGAAAAGATC AAGCTGAAGCGTTTGTAACG	(8)
vacA m	vagF vagR	AATCTGTCCAATCAAGCGAG GCGTCTAAATAATTCCAAGG	(4)
vacA s	A3436 C1226	ATGGAAATACAACAAACACAC CTGCTTGAATGCGCCAAAC	(4)
vacA i i1 i2	VacF1 C1R C2R	GTTGGGATTGGGGGAATGCCG TTAATTTAACGCTGTTTGAAG GATCAACGCTCTGATTTGA	(7)

Statistical analysis

Univariate analysis such as Chi-square test and the Fisher exact test were used to compare the association of the virulent genes with peptic ulcer diseases. Then multivariate analysis was performed to study the independency of any significant association (Genotypes with mixed status were excluded from these calculations). Significance was defined as P value of < 0.05.

Results

The presence of *H. pylori* in gastric biopsies was first detected by the CLO-test®. Those who turned negative by the CLO-test® were further tested by PCR. Out of 91 patients, 79 (86%) turned positive by either PCR or by both tests. Since each *H. pylori* isolate possesses only one single copy of *vacA* allelic variants of the s, i, or m regions, the presence of two alleles in a DNA sample indicates colonization by more than one strain (11). Overall, we found that 14/79 (17%) patients harbored mixed infections which were excluded from the statistical analysis and further study was performed on samples from 37 gastritis patients ; 23 DU patients, 3 gastric ulcer (GU) patients and 2 GC patients. The associations between virulence factors were as follows : the *cagA*+ strains were found more likely but not significantly to be associated with *vacA* s1 than s2. A 44/56 (79%) *cagA*+ strains were also *vacA* s1 positive versus 5/9 (56%) *cagA*- strains. Similarly, no significant associations were found between *cagA* status and *dupA* status. On the other hand, a significant association was detected between *cagA*+ strains 34/56 (61%) with *vacA* i1 versus 2/9 (22%) *cagA*- strains ($p < 0.04$). In univariate analysis, strains with vacA i1 genotype were significantly associated with DU and similarly in multivariate analysis these strains (*vacA* i1-type) were also independently associated with an increased odds of developing DU ($P < 0.05$, OR 3). In addition 34/49 (70%) of *vacA* s1 strains were typed as *vacA* i1 ($P < 0.001$) while only 2 of *vacA* s2 strains typed as i1). The *vacA* m2 strains were all typed as *vacA* i2 ($p < 0.001$). For GU samples, all typed *cagA*+ and vacA s1, 2/3 GU samples typed *vacA* m1 and *vacA* i1. Both GC samples were typed *cagA*+ and *vacA* i1. Because of the small sample size, the association in these samples was not determined. The amplified products, percentages of the *H. pylori* virulent genes and the significance of the statistical analysis were shown in Table 2.

Discussion

The search for *H. pylori* virulence factors predictive of specific disease is still going on. Investigators tried to link the possession of certain factors by *H. pylori* strains to the outcome of the disease and the results revealed a common consensus. The *cagA* gene and the *vacA* s1m1 are mostly detected in strains isolated from patients with severe gastric pathology (i.e. peptic ulceration and gastric cancer) rather than those with gastritis (6,11). The search continues since *H. pylori* expresses a great genetic diversity among strains distributed in different geographic locations. Patients with peptic ulceration, atrophy, intestinal metaplasia and gastric carcinoma are commonly infected with *cagA*+ strains in Western populations while in East Asian population *cagA*+ strains were common in both dyspeptic and non-dyspeptic patients (12,13). In this regard our strains are similar to those isolated from Western populations. This is also true for the *vacA* gene that contains an s region of 2 alleles s1 or s2 and an m region of 2 alleles m1 or m2 (4). The *vacA* s1m1 and s1m2 strains were also found to be associated with severe diseases in Western countries (4). It was reported that 94% of PUD patients were infected with strains that possess *cagA*+, *vacA* s1m1 or s1m2 while 25% of gastritis patients were *cagA*-[1]. In contrast to our previous study (10), significant associations were not found between *cagA*+ and *vacA* s1 and the clinical outcomes. This is probably due to the differences in the sample size between the 2 studies. Other previous studies also showed an associations between *cagA*+ stratus and the *vacA* s1 genotype (14). Rhead *et al.* (7), reported that strains with vacA s1m1 and s2m2 alleles were

Table 2. — **The percentages of the *H. pylori* virulence genes in isolates from patients with gastritis and duodenal ulcer**

***H. pylori* gene**	**Duodenal ulcer *H. pylori* + (n = 23)**	**Gastritis *H. pylori* + (n =** 37)	***P* value**
cagA+	20 (87%)	31 (84%)	1
dupA+	7 (30%)	8 (21%)	0.4
vacA s1+	19 (83%)	27 (73%)	0.5
vacA m1+	8 (35%)	8 (22%)	0.2
vacA i1+	16 (70%)	16 (44%)	0.04*

* indicates significant association.

exclusively of i1 and i2 allelic variants respectively while strains with s1m2 varied in their i-type either i1 or i2. They indicated that the i-allelic variant is an independent marker of toxicity by itself and it is associated with gastric adenocarcinoma. In another report an association was found between *vacA* i1 strains and gastric ulcer (15). In this study we also found a significant association between *vacA* i-region and DU which further substantiates the role of this factor. However in a recent study no such association was found between *vacA* i-region genotypes and the clinical outcome (16). Further studies with larger sample size from patients with different clinical outcomes are needed to determine the role of *vacA* i-region as a true virulence determinant.

Lu *et al.* (8) have examined 14 virulence genes and their association with clinical presentation, histology, and IL-8 levels and reported that *dupA* is a novel marker associated with an increased risk for DU. However, studies from Brazil, Australia and Iran showed no significant association between *dupA* prevalence and ulceration or cancer (15,17,18). In the present study, we also did not find any association between the presence of *dupA* genotype and DU. Similarly, Argent *et al.* (19) also found no significant association with DU in patients from Belgium, South Africa, China, or North America. This might be attributed to the described population differences or genetic differences between strains.

In conclusion, this study provided more evidence that the *vacA* i1 allele is one of the virulence factors of *H. pylori* significantly associated with severity of the clinical outcome. Although no significant association between cagA+ vacA s1m1 genotypes and DU was found, it indicates that the severity of the disease outcome is still dependent on several virulence factors. The search for a single predictive *H. pylori* virulence factor will continue in spite of the reported findings.

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