

## CagA and VacA genotypes in peptic ulcer disease and non-ulcer dyspepsia: a case-control study

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### Abstract

**Background:** The *cag* pathogenicity island includes a number of genes, including cytotoxin-associated protein A (*cagA*) and vacuolating cytotoxin (*vacA*) genotypes, which are associated with bacterial virulence. Although the role of *cagA* and *vacA* in the virulence of *Helicobacter pylori* (*H. pylori*) is well-established in epidemiological studies, the relationship between the *cagA* and *vacA* genotypes in Iran has yet to be fully elucidated. This study compared the association between *cagA* and *vacA* genotypes between peptic ulcer disease (PUD) patients and non-ulcer dyspeptic (NUD) patients.

**Methods:** This case control study was done on 130 patients with positive *H. pylori* in histological and Giemsa reports. The case group comprised 65 PUD patients, and the control group included 65 NUD patients. The presence of the *cagA* and *vacA* genotypes was determined using polymerase chain reaction (PCR) on biopsy samples, taken via endoscopy.

**Results:** Both *cagA* and *vacA* genotypes were positive in 51.5% (17) of the PUD group and 20% (6) of the NUD group ( $p=0.009$ ), and both *cagA* and *vacA* genotypes were negative in 48.5% (16) and 80% (24) of the case and control groups, respectively ( $p=0.03$ ). *CagA*-positive *H. pylori* was detected in 41.5% (27) and 24.6% (16) of the case and control groups, respectively ( $p=0.001$ ), and *vacA*-positive *H. pylori* was found in 60% (39) and 46% (30) of the case and control groups, respectively.

**Conclusion:** Both *cagA* and *vacA* genotypes were more prevalent in the PUD patients than in their NUD counterparts among our Iranian samples. It seems that the determination of these two genotypes in PUD patients is a good screening tool for patient selection for endoscopy and treatment.

**Keywords:** Dyspepsia, Peptic ulcer, Genes.

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### Introduction

*Helicobacter pylori* (*H. pylori*) is a Gram-negative spiral-shaped microaerophilic bacterium that colonizes the human gastric mucosa (1). It is estimated that the prevalence of *H. pylori* infection ranges between 20% and 80% in the world (2,3). Although not all persons infected with this organism develop gastroduodenal pathology (4), *H. pylori* is now well-recognized as the major etiologic agent of chronic gastritis, peptic

ulcer disease (PUD), adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (MALToma) (5,6).

Several virulence factors have been recognized so far. However, cytotoxin-associated protein A (*cagA*) and vacuolating cytotoxin (*vacA*) are known to have great potential to cause disease development. *CagA* is a highly immunogenic protein of 128 kDa, and *vacA* is a protein of 87 kDa which can vacuolize the gastric epithel-

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lial cells. These proteins are produced by some *H. pylori* strains which are encoded by the *cagA* and *vacA* genes. Half of all cytotoxin-positive *H. pylori* strains have the *cagA* gene in their genome (7,8).

Previous studies have shown that strains which have *cagA* and produce cytotoxin protein are closely associated with PUD, albeit with different geographical prevalence rates (9- 11). Furthermore, the *vacA*-positive strain of *H. pylori* is known to be associated with PUD (12,13). Xiang et al. classified *H. pylori* strains in two main groups: type I strain had the gene coding for *cagA* and expressed *cagA* and *vacA* and type II strain did not have the gene coding for *cagA* and expressed neither *cagA* nor *vacA*. They reported rates of 56% and 16% for strain types I and II, respectively (9). Weel et al. demonstrated that *H. pylori* strains with the *cagA* and *vacA* genotypes were positive in 56.6% and 31.6% of patients with PUD and functional dyspepsia, respectively (12). Takata et al. found both *cagA* genotype and anti-*vacA* antibody in 62.9% and 7.8% of PUD and non-ulcer dyspeptic (NUD) patients, respectively (10). Elsewhere, Figueroa et al. reported that both anti *cagA* and *vacA* antibodies existed in 85% and 71% of patients with PUD and NUD, respectively (11). Moreover, Maeda et al. reported that there were both anti-*cagA* and *vacA* antibodies in 81%, 82%, and 72% of patients with gastric ulcer, duodenal ulcer, and NUD, respectively, thereby demonstrating that there was no significant difference between PUD and NUD groups (14).

For all the research in epidemiological studies into the role of the *cagA* and *vacA* genotypes in the virulence of *H. pylori* around the world, precious little information is available on the expression of these two genotypes in the *H. pylori* strain in Iran. The present study sought to compare the expression of *cagA* and *vacA* *H. pylori* virulence factors between a PUD group and an NUD group.

## Methods

The study population was selected from dyspeptic patients admitted to Firoozgar Hospital, Tehran, Iran. According to the Leeds Medical School Criteria (15), 802 patients who had dyspeptic symptoms at least for two months with no underlying diseases were initially selected. All the patients provided informed consent and accepted to complete a standard questionnaire. Diagnosis was based on questionnaires and esophagogastroduodenoscopy (Fujinon ED-53 DEP) results. From this total, a case-control study was conducted on 130 patients, aged between 15 and 65 years. The case group was comprised of 65 PUD patients, and the control group consisted of 65 NUD patients. The case and control groups had no history of documented ulcer, previous *H. pylori* eradication, cigarette smoking, malignancy, underlying diseases, or esophagitis in esophagogastroduodenoscopy, and nor did they use proton pump inhibitors or antibiotics (from at least two months before endoscopy).

Peptic ulcer development depends on age and gender; accordingly, all the selected patients were matched accurately in terms of their age and gender. Four biopsy specimens were taken from the antrum and gastric body of each patient for histological study. All the specimens were stained by hematoxylin and eosin stain (H&E) or Giemsa after having been fixed overnight in buffered formalin, embedded in paraffin, and cut in five  $\mu\text{m}$  thickness. The specimens were evaluated by a pathologist. If at least five bacilli in each microscopic field were found, *H. pylori* was considered positive. Five milliliters of blood sample was taken from each patient and was sent to the Immunology Laboratory of Iran University of Medical Sciences for centrifuge and anti-*cagA* antibody measurement.

The genomic DNA was extracted from the biopsy samples using a DNA isolation kit for cells and tissues (Roche Applied Science Company) in accordance with the Manufacture's instruction and stored at  $-20^{\circ}\text{C}$ . Two primers were designed comple-

Table 1. Characteristics of the primers used for the detection of *cagA* and *vacA*

Amplified region	Primer designation	Primer sequence	Product size ( bp )
GlmM	GlmM-F	5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3'	294
	GlmM-R	5'-AAGCTTACTTTCTAACACTAACGC-3'	
CagA1	CAG1-F	5'-GAT AAC AGG CAA GCT TTT GAG G-3'	349
	CAG1-R	5'-CTG CAA AAG ATT GTT TGG CAG A-3'	
CagA2	CAG2-F	5'-TTG ACC AAC AAC CAC AAA CCG AAG-3'	1385
	CAG2-R	5'-CTT CCC TTA ATT GCG AGA TTC C-3'	
VacA1	VAC1 - F	5'-CTG CTT GAA TGC GCC AAA C-3'	71
	VAC1 -R	5'-CAC AGC CAC TTT CAA TAA CGA-3'	
VacA2	VAC2 - F	5'-ATG GAA ATA CAA CAA ACA CAC-3'	480
	VAC2 - R	5'-CGT CAA AAT AAT TCC AAG GG-3'	

Bp, base pair; GlmM, urea C gene used for the detection of *H. pylori*; F, forward; R, reverse; CagA1 and A2, two pairs of primers used for the detection of *cag*, which are called A1 and A2; VacA1 and A2, two pairs of primers used for the detection of *vac*, which are called A1 and A2

mentary to the sequence located within the conserved region of the gene primers: *cagA1* and *cagA2*. The primers F and R and their sequences as well as the sizes of the PCR products are depicted in Table 1.

The amplified products were evident as ethidium bromide-stained bands after Agarose gel electrophoresis. Moreover, 349 bp band indicated the presence of the *H. pylori cagA* in the specimen, as is shown in Figure 1. The identification of the bacilli as *H. pylori* was confirmed via PCR for GlmM, a conserved gene formerly known as urea C (Fig. 2) (16).

The identification of *vacA* gene was confirmed by PCR using primers specific for its signal sequence with the pair of primers: *vacA1* and *vacA2*. The characteristics of the primers are illustrated in Table 1. The amplified product was evident as above, and 480 bp band indicated the presence of

the *H. pylori vacA* in the specimen, as is shown in Fig. 3.

All the data were analyzed using SPSS software (version 18) after encoding for each patient. Age is shown with age  $\pm$  standard deviation. The effects of Cag A and Vac A positive on the risk peptic ulcer developed were expressed as Odds ratios (ORs) with 95% confidence intervals (CIs) with reference of NUD subjects with *H. Pylori* infections. Proportions were compared by Fisher exact probability test and the chi-squared test. A P-value less than 0.05 was considered statistically significant

## Results

On the basis of the Leeds Medical School Criteria, the case group included 65 PUD patients [including 37 (57%) females and 28 (43%) males at an average age of  $41.6 \pm 16.4$  years (16 to 64)]. In the case group, gastric ulcer was detected in 27 (41.5%) patients [21 (77.8%) females and 6 (22.2%) males] and duodenal ulcer was found in 38 (58.5%) patients [16 (42.1%) females and 22 (57.9%) males]. The control group (NUD) group included 65 patients, comprised of 29 (45%) females and 36 (55%) males at an average age of  $36.4 \pm 10.8$  years (18 to 60).

The overall frequency of the *cagA* gene was 43/130 (33%): 27/65 (41.5%) in the case (PUD) group and 16/65 (24.6%) in the control (NUD) group ( $p=0.001$ ). The total frequency of the *vacA* gene was 69/130 (53%): 39/65 (60%) in the PUD group and 30/65 (46%) in the NUD group ( $p=0.25$ ).

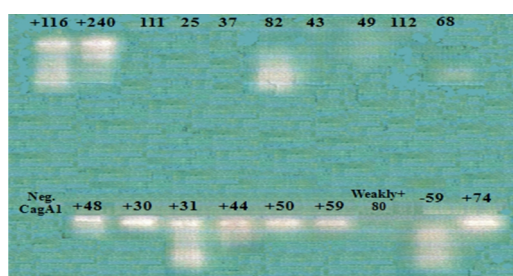
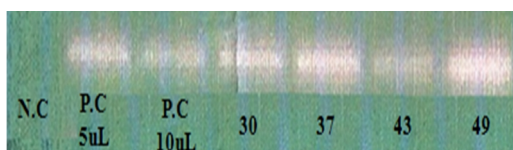


Fig. 1. CagA-positive and negative samples

Fig. 2. Confirmation of *H. pylori* by the demonstration of the urea C gene on Agar electrophoresis

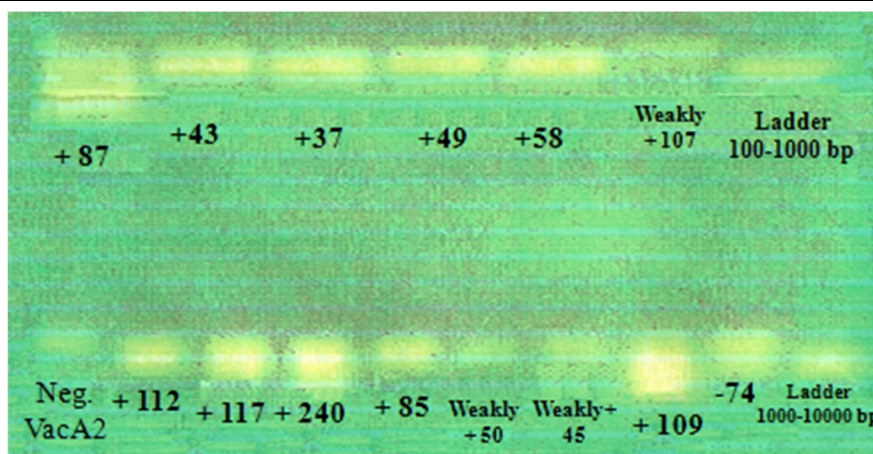


Fig.3. VacA-positive and negative samples

Table 2. Relation between endoscopic findings and genotypes

Genotype	Number of cases (%)		p
	(Case)PUD	(Control)NUD	
CagA+VacA+	17(51.5)	6(20)	0.009
CagA-VacA-	16(48.5)	24(80)	0.03

PUD, peptic ulcer disease; NUD, non-ulcer dyspepsia; CagA, cytotoxin-associated protein A; VacA, vacuolating cytotoxin A; +, positive; -, negative

The *cagA* and *vacA* genes were both positive in 17/33 (51.5%) and 6/30 (20%) of the patients in the case and control groups, respectively. The rate was, therefore, significantly higher in the case group than in the control group (odds ratio: 4.25, 95%CI: 1.25-13.06;  $p=0.009$ ). These results are presented in Table 2.

### Discussion

To the best of our knowledge, this study is the first Iranian case-control study to date to examine the relationship between both *cagA* and *vacA* genotypes of *H. pylori* in patients with or without PUD. Our results showed that 51.5% of the patients in the case (PUD) group had both *cagA* and *vacA* genotypes and that this rate was higher than that (20%) in the control (NUD) group. Although smokers and patients receiving acid-inhibiting medications or nonsteroidal anti-inflammatory drugs were excluded from this study, the results were similar to those reported by previous studies (9, 10 and 12). Therefore, this could be a reliable method for the diagnosis of PUD. Our results also demonstrated a correlation between the two genotypes of *H. pylori*, i.e., *cagA* and *vacA*, and PUD.

In contrast to the virtual absence of any

research in Iran into the expression of the two genotypes of *H. pylori* strains, the literature abounds with information elsewhere. In 1995, Xiang et al. classified *H. pylori* strains in two main groups: type I strain had the gene coding for *cagA* and expressed *cagA* and *vacA* and type II strain did not have the gene coding for *cagA* and did not express either *cagA* or *vacA*. Additionally, the authors reported rates of 56% and 16% for types I and II, respectively (9). In 1996, Weel et al. showed both *cagA* and *vacA*-positive *H. pylori* strains in 56.6% and 31.6% of patients with PUD and functional dyspepsia, respectively (12). In 1998, Takata et al. found both *cagA* genotype and anti-*vacA* protein antibody in 62.9% and 7.8% of patients with PUD and NUD, respectively (10). In 2002, Figueroa et al. reported both anti *cagA* and *vacA* protein antibodies in 85% and 71% of patients with PUD and NUD, respectively; they believed that these results were due to the high prevalence of both *cagA* and *vacA*-positive *H. pylori* strains in their country (11). In 1998, Maeda et al. demonstrated both anti-*cagA* and *vacA* antibodies in 81%, 82%, and 72% of patients with gastric ulcer, duodenal ulcer, and NUD, respectively, and concluded that the difference

between the PUD and NUD groups was not significant (14).

These results can be explained by the high prevalence of type I strain of infection in Japan.

Most of the studies in this field have measured serum anti-cagA and vacA antibodies or vacA activity in vitro or in patients. Some studies have shown that the vacA activity and protein can occur even when the cagA or vacA genotypes cannot be detected and that there are some strains that can produce inactive or small amounts of the vacA protein. The use of gene-specific primers in epidemiological studies is the best method to demonstrate the cause and effect of the virulence factors in infectious diseases because the vacA protein can be detected in the absence of the expression of the cagA and vacA genotypes or because some strains can produce small amounts of this protein (9- 11 and 14).

There have been some controversies with respect to the association between the cagA and vacA genotypes and gastrointestinal disorders in Iranian studies (17-23). Where most of these investigations have reported that the cagA gene is more prevalent in Iran than in European and Western countries (17,18), Siavoshi et al. showed that only 44% of *H. pylori* strains are cagA positive (19), which chimes in with our findings. In addition, some Iranian reports have demonstrated a relationship between the cagA genotype and gastrointestinal disorders (20,21), whereas others have detected no correlation (22,23).

It has been demonstrated that *H. pylori* carries only a single copy of the vacA gene. The risk of *H. pylori* with multiple strains may be higher in countries with high prevalence rates of *H. pylori* infection than in those with low prevalence rates (9,14).

Several studies have demonstrated that patients with duodenal ulcer are most often infected by strains which have the cagA and vacA genotypes. Consequently, it has been suggested that both virulence factors play a role in the pathogenicity of peptic ulcers (9). It has also been reported that on-

ly strains with both cagA and vacA genotypes can induce gastric injury in the mice (13).

The present study has some limitations, first and foremost among which is that it did not assess factors such as the study population's socioeconomic status, diet, and immune system. In addition, other virulence factors of *H. pylori* were not studied and there was referral bias in patient selection (14).

### Conclusion

The present study showed that both *H. pylori* cagA and vacA genotypes were more prevalent in the PUD group than in the NUD group in our sample of Iranian patients. We would suggest that other gastrointestinal diseases be tested for both genes using gene-specific primers among Iranians, because the characteristics of a host's immune response or environmental factors may also play important roles in the pathogenesis of these diseases. It seems that the assessment of both genotypes in PUD patients is a better way of selecting patients with dyspepsia for endoscopy and treatment.

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