Gastroduodenal pathology in the light of *Helicobacter pylori* genotype in Egyptian patients

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Received: 10 July 2019
Accepted: 6 September 2019
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:550–555

Context
Infection with *Helicobacter pylori* is associated with gastroduodenal diseases such as gastritis, gastric ulcer, duodenal ulcer, gastric cancer, and mucosa-associated lymphoid tissue lymphoma.
Aim
The aim of this study was to detect the nature of gastroduodenal pathology in the light of the genotype of the associated *H. pylori* organism.
Materials and methods
The study was conducted on 100 patients with upper gastrointestinal tract symptoms; infection with *H. pylori* was detected by stool antigen test. Moreover, 20 asymptomatic patients, infected with *H. pylori*, were included in the study as controls.
Upper gastrointestinal tract endoscopy was performed in all participants to take biopsies to diagnose the disease microscopically and to determine *H. pylori* virulence factors [cytotoxin-associated protein A (CagA) and VacA] by PCR.
Results
Patients infected by *H. pylori* organisms having CagA-positive genes (41 patients) developed gastritis in 53.7%, peptic ulcer disease (PUD) in 36.6%, and gastric malignancy in 9.8%. Patients infected with organisms that have VacA s1 in addition to CagA genes (19 patients) were found to have gastritis in 21.1%, PUD in 63.2%, and gastric malignancy in 15.8%. However, patients infected with *H. pylori* organism that have VacAs2 in addition to CagA genes (34 patients) developed gastritis in 79.4%, PUD in 20.6%, and no malignancy.
Discussion
The presence of VacA s1 gene in addition to CagA significantly increases the virulence of the organism toward development of PUD and gastric malignancy. The presence of VacA s2 gene significantly decreases the virulence of CagA gene to develop PUD and prevent completely its carcinogenicity.

Keywords:
CagA, gastric malignancies, gastroduodenal diseases, *H. pylori*, PUD, VacA

Egypt J Intern Med 31:550–555

1110-7782

Introduction

Helicobacter pylori is a Gram-negative spiral-shaped microorganism ~3 μm long with a diameter of ~0.5 μm and has 4–6 flagella that make it highly motile [1]. It is currently estimated that approximately half of the world population is infected with the *H. pylori* [2].

H. pylori colonizes the gastric epithelium of the human stomach early in life (<10 years of age) and remains latent in most infected patients [3,4]. It becomes symptomatic later in life when the infection results in development of gastroduodenal disease. The frequency and severity of disease outcome differs between infected patients and depends on interplay between the virulence of the infecting strain of the bacterium, the type and severity of the patients' immune response, and environmental factors [5].

H. pylori strains possess specific virulence factors that play an important role in the development of gastritis, peptic ulcer diseases (PUD), gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma [6]. One of these virulence factors is the cytotoxin-associated protein A (CagA protein) encoded by the cytotoxin-associated gene (CagA gene) present in the genome of *H. pylori*. This toxic protein when gains access to gastric epithelial cells becomes active through its phosphorylation on tyrosine residues by the host cell kinase. It stimulates cell signaling pathways leading to cytoskeletal changes, epithelial cell proliferation, or cell apoptosis, which if not well regulated may lead to malignant diseases or

ulcers [7]. It may also disrupt apical junctional complexes between epithelial cells leading to loss of barrier function [8]. CagA protein may have between 0 and 5 active tyrosine phosphorylation sites. More sites lead to high levels of CagA phosphorylation in epithelial cells [9].

The other virulence protein is vacuolating cytotoxic protein (VacA protein) encoded by the vacuolating cytotoxic gene A (VacA gene) which is present in the genome of all *H. pylori* strains. The mature secreted VacA protein is polymorphic with variable cytotoxic activity. The toxin is composed of the following regions: the signal peptide region (s) and the intermediate or binding region (m). The signal peptide region is of two types, s1 or s2; s1 type is active and s2 type is inactive. The binding region is also of two types, m1 or m2; m1 type is active and m2 type is less active. VacA protein may comprise any combination of s and m types. Type s1m1 VacA is fully active, and type s1m2 is active but binds to narrower range of cells; type s2m1 and s2m2 do not induce vacuolation [10–12].

Materials and methods

This study was conducted on 100 Egyptian patients having upper gastrointestinal symptoms and infected with *H. pylori* documented by stool antigen test [13]. They were subjected to upper gastrointestinal endoscopy to diagnose any associated lesion and take biopsy specimens for microscopic examination [14].

Moreover, 20 patients having positive *H. pylori* Ag in stool and asymptomatic were included as controls.

Exclusion criteria

The following were the exclusion criteria:

(1) Patients aged above 70 years old.
(2) Patients with hepatic failure.
(3) Patients with renal failure.
(4) Patients with cardiac failure.
(5) Patients with bleeding and/or coagulation disorders.
(6) Patients taking antisecretory drugs.
(7) Patients receiving anti-*H. pylori* antibiotic or bismuth in the prior 6 months.

An informed consent was obtained from all participants, and the study was carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki).

All of the participants included in the study were subjected to the following:

(1) Full history taking, with particular stress on the following:
   (a) Analysis and duration of the complaint (epigastric pain, heartburn, dyspepsia, vomiting, hematemesis, melena, and loss of weight).
   (b) History of smoking, drug intake, and any associated diseases.
   (c) History of any operations and/or procedures and complications if present.
(2) Complete clinical examination.
(3) Laboratory investigations: routine investigations including complete blood count, fasting blood sugar, renal function tests, liver enzymes, and prothrombin time and activity.
(4) Upper gastrointestinal endoscopy: diagnostic upper gastrointestinal endoscopic examination was done for all participants, and the endoscopic findings were recorded. From these patients, gastric biopsy specimens were taken from the antrum within 2 cm of the pyloric channel for rapid urease test (one tissue biopsy), PCR assay (one tissue biopsy), microbiological examination (three fragments), and histopathological examination (three fragments).
   Endoscopic findings were divided into three categories: nonerosive gastritis, PUDs, and gastric malignancies.
(5) PCR examination includes the following [15]:
   (a) DNA extraction.
   (b) PCR amplification for detection of *H. pylori* DNA.
   (c) Multiplex PCR to detect *H. pylori* CagA and VacA genes.
   (d) Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois, USA).

Cross tabulation, frequency tables, and χ^2-tests were derived to detect association between different variables under study.

Results

Demographic data

Demographic data are presented in Table 1.

Types of *H. pylori*: by using tissue PCR, *H. pylori* strains were categorized into three types: type I expressing both CagA and VacA, type II expressed neither CagA nor VacA, and type III expressing CagA only (Table 2).

The incidence of CagA and VacA genotypes in symptomatic (cases) and asymptomatic patients (controls) is illustrated in Fig. 1.

The incidence of gastroduodenal pathology in CagA only genotype

The incidence of gastroduodenal pathology in CagA only genotype is illustrated in Figs 1 and 2 (Table 3).

The incidence of gastroduodenal pathology between CagA only genotype and CagA associated with VacAs1 genotype

The incidence of gastroduodenal pathology in CagA, VacA s1 genotype is illustrated in Fig. 3 (Table 4).

The incidence of gastroduodenal pathology between CagA only genotype and CagA associated with VacAs2 genotype

The incidence of gastroduodenal pathology in CagA associated with VacA s2 genotype is illustrated in Fig. 4 (Table 5).

Table 1 Age and sex distribution among cases and controls

	Age (mean±SD)	Sex [*n* (%)]	
		Male	Female
Cases			
Range	18.0–65.0	45 (45)	55 (55)
Mean±SD	38.4±12.39		
Controls			
Range	18.0–44.0	11 (55)	9 (45)
Mean±SD	29.5±6.62		
Tests	t=4.61	χ^2=0.67	
P value	<0.001*	0.413	

*$P \leq 0.05$, statistically significant.

Discussion

Analysis of demographic data revealed no significant difference between cases and controls in sex distribution, whereas there was a significant difference regarding age. These results were in accordance with those found in developed and developing countries, where it was found that increasing age was associated with increasing risk of *H. pylori* infection [16].

H. pylori strains differ, and possession of specific virulence factors greatly increases the risk of the disease. The best recognized of these are CagA and VacA proteins.

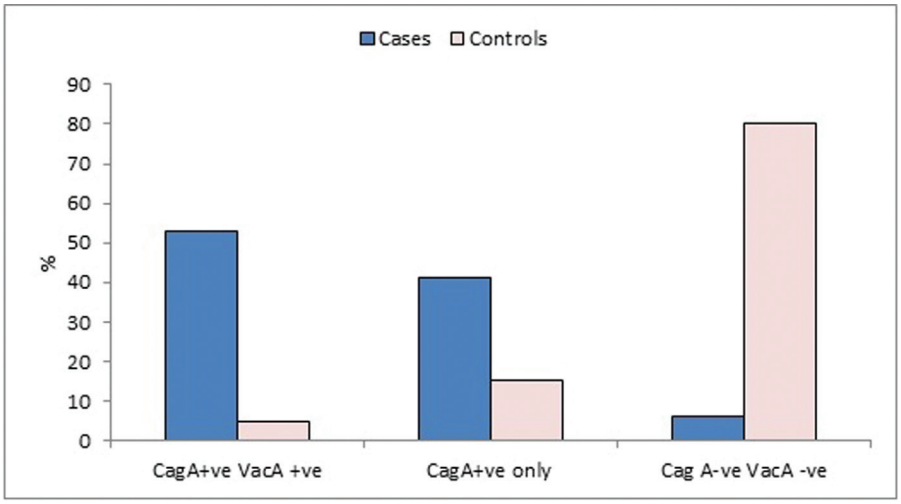
Based on the analysis of expression of these two virulence factors, isolates of *H. pylori* were divided into four genotypes: genotype expressing CagA only in 41 (41%) patients, genotype expressing CagA in addition to VacAs1 in 19 (19%) patients, CagA VacA s2 genotype in 34 (34%) patients, and genotype lacking both CagA and VacA in six (6%) patients.

Table 2 Categories of *H. pylori* strains

	CagA positive VacA positive [*n* (%)]	CagA positive only [*n* (%)]	CagA negative VacA negative [*n* (%)]	Total [*n* (%)]
Cases	53 (53.0)	41 (41.0)	6 (6.0)	100 (100.0)
Controls	1 (5.0)	3 (15.0)	16 (80.0)	20 (100.0)
Test (*P*)	χ^2=61.388 (<0.001)*			

*Most prevalent genotype among patients.

Figure 1



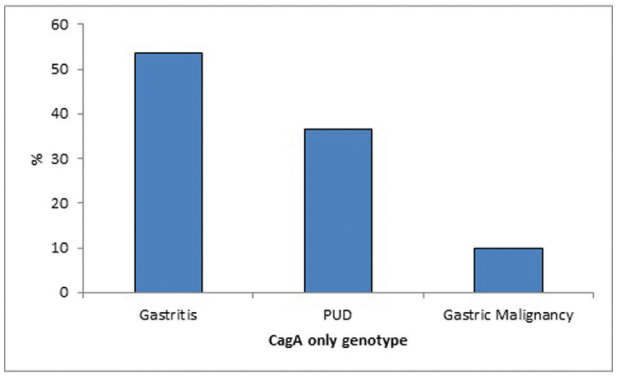
The incidence of cytotoxin-associated protein A and vacuolating cytotoxic gene A genotypes in symptomatic (cases) and asymptomatic patients (controls).

Although much is written in world literature about the frequency and pathogenicity of CagA genotype, little is written about the frequency and pathogenicity of the other three genotypes.

In USA, the prevalence of CagA genotype was 60% [17]. In Europe, its prevalence is 62% in Spain [18] and 68%in England [19]. In Asia, its prevalence was usually over 85% in Japan, Korea, and China [20,21]. In the Middle East, its prevalence is 71% in Iraq, 78% in Turkey, and 76% in Iran [22,23].

In Jordan, the CagA genotype was detected in 26.4% [24], whereas Kuwaitis and other Arabian Gulf Arabs had essentially the same prevalence rate of ~41% [25]. In a study conducted in Israel, CagA genes were present in only 25.5% [26]. The prevalence of CagA positivity in Saudi Arabia was 52% [27].

Figure 2



The incidence of gastroduodenal pathology in cytotoxin-associated protein A –only genotype.

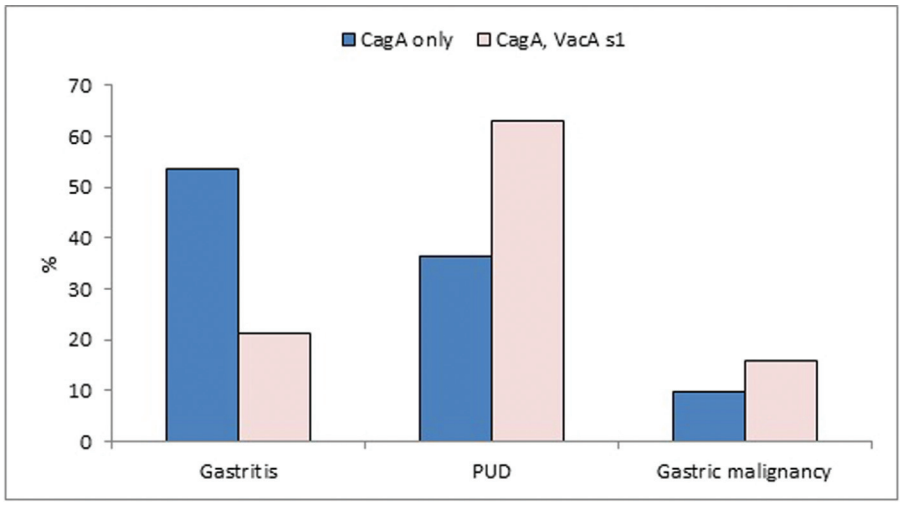
In this study, CagA genotype was isolated in 41 (41%) patients: 22 (53.7%) patients had gastritis, 15 (36.6%) patients had PUD, and four (9.8%) patients had gastric malignancies. These results are in agreement with many studies [28,29].

The mosaic structure of VacA represents an important factor in variation of its vacuolating cytotoxin activity. In general, type s1 produce high and moderate levels of toxin activity, whereas s2 strains produce no vacuolating activity. Heterogeneity among VacA alleles may be an important factor in understanding variations in pathogenicity among *H. pylori*-infected participants [30,31].

The pathogenicity of VacA in association with CagA genome in the same organism was rarely studied in world literature. In the present study, this genotype was found in 53 (53%) patients, where 19 patients had CagA VacAs1 genotype and 34 patients had CagA VacAs2 genotype.

In the present study, the presence of VacAs1 in association with CagA (CagA VacA s1) increased the toxicity of CagA significantly. In comparison with CagA genotype, PUD incidence was increased from 36.6 to 63.2%, and the incidence of gastric malignancy was increased from 9.8 to 15.8%. In contrast, the association of VacA s2 with CagA genome (CagA VacAs2) decreased the toxicity of CagA genotype. In comparison with CagA genotype, PUD decreased significantly from 36.6 to 20.6%, and the incidence of malignancy was 0% compared with 9.8%. This is explained by the presence of 12-amino-acid hydrophilic amino-

Figure 3



The incidence of gastroduodenal pathology in cytotoxin-associated protein A associated with vacuolating cytotoxic gene A s1 genotype.

terminal segment, present in type s2 but absent from type s1 VacA proteins; this segment slows the capacity of VacA to form membrane channels and abolishes vacuolation.

Particular VacA genotypes have been considered markers for the pathogenicity of individual *H. pylori* strains, as production of cytotoxin *in vitro*, epithelial damage *in vivo* and the development of PUD are related to specific VacA genotypes. The elevated toxicities of s1 strains may contribute to the development of ulcerations. Indeed, VacA s1 strains secrete larger amounts of cytotoxin than VacA s2 strains *in vitro*, the latter supposedly being less virulent. Indeed, carrying *H. pylori* with the VacA s1 genotype significantly increased the risk of peptic ulcer or gastric cancer compared with gastritis alone [26,32].

H. pylori organism lacking both CagA and VacA (CagA negative and VacA negative) can be present in the stomach, causing no lesion. This was found in 80%% of the controls (16 out of 20 participants) and 6% of patients (6 out of 100). This can explain that most *H. pylori* infected participants are asymptomatic.

Table 3 The incidence of gastroduodenal pathology in CagA only genotype

Genotype	Number of patients	Gastritis	PUD	Gastric malignancy
CagA only	41	22 (53.7%)	15 (36.6%)	4 (9.8%)

PUD, peptic ulcer disease.

Conclusion

Patients with CagA positive genotype but Vac A negative are prone to develop PUD in 36.6% and gastric malignancies in 9.8% of cases.

(1) The association of VacAs1 with CagA increases significantly the toxicity of CagA. Patients are prone to develop PUD (≥63.2%) and gastric malignancies (≥15.8%).

Table 4 The comparison between the incidence of gastroduodenal pathology between cytotoxin-associated protein A-only genotype and cytotoxin-associated protein A associated with vacuolating cytotoxic gene A s1 genotype

Genotype	Number of patients	Gastritis [*n* (%)]	PUD [*n* (%)]	Gastric malignancy [*n* (%)]
CagA only	41	22 (53.7)	15 (36.6)	4 (9.8)
CagA, VacA s1	19	4 (21.1)	12 (63.2)	3 (15.8)
Test (*P* value)			χ^2=5.6 0.049*	

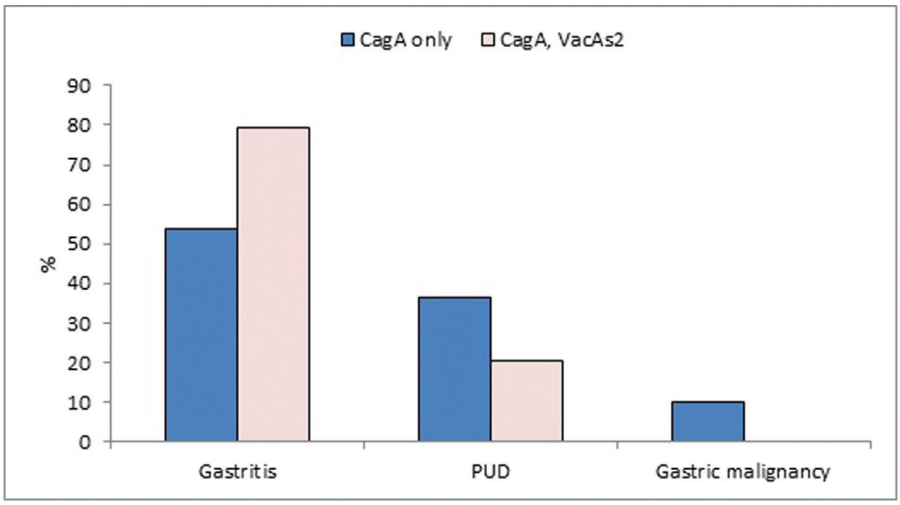
CagA, cytotoxin-associated protein A; PUD, peptic ulcer disease; VacA, vacuolating cytotoxic gene A.

Table 5 The comparison between the incidence of gastroduodenal pathology between cytotoxin-associated protein A-only genotype and cytotoxin-associated protein A associated with vacuolating cytotoxic gene A s2 genotype

Genotype	Number of patients	Gastritis [*n* (%)]	PUD [*n* (%)]	Gastric malignancy [*n* (%)]
CagA only	41	22 (53.7)	15 (36.6)	4 (9.8)
CagA, VacAs2	34	27 (79.4)	7 (20.6)	0 (0)
Test (*P* value)			χ^2=6.1 0.042*	

CagA, cytotoxin-associated protein A; PUD, peptic ulcer disease; VacA, vacuolating cytotoxic gene A.

Figure 4



The incidence of gastroduodenal pathology in CagA associated with vacuolating cytotoxic gene A s2 genotype.

(2) The presence of s2 with CagA decreases significantly the toxicity of CagA. The patients are prone to develop PUD in only 20.6% of patients with no malignancy.
(3) Patients with CagA-negative and VacA-negative strains are rarely prone to develop gastroduodenal pathology.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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