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Relationship between *Helicobacter pylori* Virulence Genes and Clinical Outcomes in Saudi Patients

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Helicobacter pylori has been strongly associated with gastritis, gastric and duodenal ulcers, and it is a risk factor for gastric cancer. Two major virulence factors of *H. pylori* have been described: the cytotoxin-associated gene product (*cagA*) and the vacuolating toxin (*vacA*). Since considerable geographic diversity in the prevalence of *H. pylori* virulence factors has been reported, the aim of this work was to determine if there is a significant correlation between different *H. pylori* virulence genes (*cagA* and *vacA*) in 68 patients, from Saudi Arabia, and gastric clinical outcomes. *H. pylor* was recognized in cultures of gastric biopsies. *vacA* and *cagA* genes were detected by polymerase chain reaction (PCR). The *cagA* gene was obtained with 42 isolates (61.8%). The *vacA* s- and m- region genotypes were determined in all strains studied. Three genotypes were found: s1/m1 (28%), s1/m2 (40%) and s2/m2 (26%). The s2/m1 genotype was not found in this study. The relation of the presence of *cagA* and the development of cases to gastritis and ulcer was statistically significant ($P < 0.05$). The study showed a significant correlation between the *vacA s1/m2* genotype and gastritis cases, and a significant correlation between *vacA s1/m1* genotype and peptic ulcer cases. The results of this study might be used for the identification of high-risk patients who are infected by *vacA s1/m1* genotype of *H. pylori* strains. In conclusion, *H. pylori* strains of *vacA* type s1 and the combination of s1/m1 were associated with peptic ulceration and the presence of *cagA* gene.

Key Words: *Helicobacter pylori*; *cagA*; *vacA*; Gastritis; Peptic Ulcer

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative spiral bacterium which colonizes the human stomach (1). Infections with *H. pylori* may induce chronic gastritis, peptic ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma (MALT) (2). There is increasing evidence that the genetic variability of *H. pylori* may have a clinical importance (3). There are two bacterial virulence factors of *H. pylori* and their genes also serve as epidemiological markers. The *vacA* (vacuolating toxin) and the *cagA* (cytotoxin-associated gene) play a major role in determining the clinical outcome of *Helicobacter* infections (4). The *vacA* gene, encoding the vacuolating toxin, is considered an important virulence factor and it is present in all strains (5). Significant sequence polymorphisms within *vacA* can be found in the coding sequence for the signal peptide (referred to as the s-region) and in the middle of the gene, called the middle (m) region. There are two allelic types (*m1* and *m2*) in the middle region while the signal region has either an s1 (*s1a*, *s1b*, and *s1c*) or an *s2* allele. The strains of the *s1/m1* subtype typically produced higher levels the vacuolating cytotoxin than other genotypes, while *s2/m2* strains do not secrete *vacA* (6). Toxins with different m- genotypes also display a differential specificity for intoxicating the target mammalian cells, with *vacA* m1 variants affecting wider range of target cells than those with m1 (7).

The product of the *cagA* gene is introduced into gastric epithelial cells by the type IV secretion system, where it becomes phosphorylated and modulates various cellular processes and signal transduction pathways. The intracellular *cagA* activities associated with the development of gastric carcinoma include disruption of tight junctions and a the responses (8). The presence of the *cagA* gene is an important marker for the most virulent strains associated with peptic ulcer, atrophic gastritis and adenocarcinoma (9). The *cagA* gene is a marker for the presence of the *cag* pathogenicity island and this gene with others on the island is associated with more severe clinical outcomes (10, 11). Atherton et al. (6) first reported a strong association between *cagA* and *vacA* signal sequence type *s1/m1*.

The patterns of *H. pylori* genotypes have been recently analyzed in several patient populations worldwide (6, 12-18). However, no such study has been carried out in Saudi Arabia. Here I report the results of such study and for the first time, investigate the prevalence of the *cagA* and *vacA* genotypes of *H. pylori* iso-

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lates from gastric cultures and demonstrate their relationships with clinical outcomes.

MATERIALS AND METHODS

Biopsy samples were obtained over a 6 months-period (January through June 2010) from selected patients referred for endoscopy at different hospitals in Riyadh, Saudi Arabia. Sixty eight patients, who had *H. pylori*, were enrolled in this study. The mean age of the patients was 43 yr (range, 14-84) and 45% were female. Histologically, patients were classified into gastritis in 37 cases (54.4%) and peptic ulceration in 31 cases (45.6%). One biopsy specimen taken from the antrum was used for the culture. All data of the subjects were collected from patient's file.

H. pylori culture and DNA extraction

Antral biopsies were cut into small pieces, homogenized and were smeared on the surface of *H. pylori* selective agar (Oxoid, Basingstoke, Hants, UK) then incubated at 37°C in a BBL GasPak (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) containing a Campy-Pak Plus microaerophilic system generator (Becton Dickinson Microbiology Systems) for 7 days. The morphology of *H. pylori* clinical isolates colonies were smooth, translucent and small. Colonies that manifested the described characteristic morphologies were identified as *H. pylori* if they were Gram negative and shaped bacilli, and urease, catalase and oxidase positive. From the primary growth, seven or eight colonies were pooled together, and genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to instructions of the manufacturer. The isolated DNA was eluted in 200 μL of 1 × TE buffer (10 mMTris-HCl, 1 mM EDTA [pH 8.0]) and stored at -20°C until use.

H. pylori genotyping for *cagA* and *vacA*

After DNA samples extraction, polymerase chain reactions (PCR) were carried out in a volume of 50 μL containing 1 μM of each primers, 1 μL of genomic DNA (approximately 200 ng), 1 mM of dNTPs mix, 2 mM of $MgCl_2$, and 0.05 U/μL *Taq* DNA polymerase.

PCR amplifications were carried out in GeneAmp PCR system 9700 (Perkin Elmer, Norwalk, CT, USA). Table 1 summarized the primer sequences and the expected size of PCR products. The following cycle conditions were used: for *cagA*: 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C and for *vacA*: 35 cycles of 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C. All runs included one negative and one positive DNA control. A 10 μL of amplified PCR products was then resolved by electrophoresis on 1.5% agarose gels run in acetate EDTA buffer and stained with ethidium bromide. The PCR product was visualized under a short wave length ultraviolet light source (Fig. 1).

Table 1. PCR primers for amplification of *cagA* and *vacA* sequences (19)

Region	Primer	Primer sequence (5′-3′)	PCR product size (bp)
cagA	F1	GATAACAGCCAAGCTTTTGAGG	349
	B1	CTGCAAAAGATTGTTTGGCAGA	
vacA			
m1	VA3-F	GGTCAAAATGCGGTCATGG	290
	VA3-R	CCATTGGTACCTGTAGAAAC	
m2	VA4-F	GGAGCCCCAGGAAACATTG	352
	VA4-R	CATAACTAGCGCCTTGCAC	
s1/s2	VA1-F	ATGGAAATACAACAAACACAC	259/286
	VA1-R	CTGCTTGAATGCGCCAAAC	
s1b	SS3-R	AGCGCCATACCGCAAGAG	187
	VA1-R	CTGCTTGAATGCGCCAAAC	
s1a	SS1-R	GTCAGCATCACACCGCAAC	190
	VA1-R	CTGCTTGAATGCGCCAAAC	
s2	SS2-R	GCTAACACGCCAAATGATGC	199
	VA1-R	CTGCTTGAATGCGCCAAAC	

Data analysis

Fisher's exact test was used for analysis of data. A P value of < 0.05 was accepted as statistically significant.

Ethics statement

Written informed consent was obtained from each participant before endoscopy.

RESULTS

Prevalence of *cagA* and *vacA* genotyping

The *cagA* gene was obtained with 42 isolates (61.8%) and 26 (38.2%) were negative. The *vacA* s- and m- region genotype were determined in all strains studied. For the s-region, in strains where a single *vacA* s allele was found, the minority 18 (26%) contained the *s2* allele. In 46 isolates contained *s1* allele (68%), 23 (34%) were subtype for each *s1a* and *s1b*. In the m-region, 2 strains contained both *m1* and *m2* alleles. In the strains containing one single *vacA* m allele, the *m1* allele was found in 19 isolates (28%) and *m2* in 45 ones (66%). Considering strains with

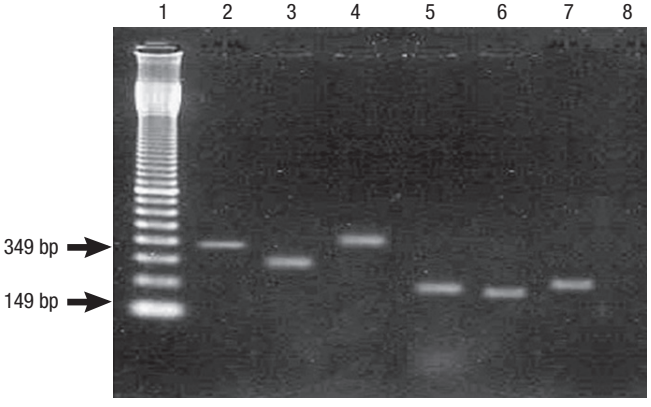

Fig. 1. PCR genotyping of *vacA* and *cagA* status from different cases. Primers described in Table 1 were used for PCR reaction (Lanes- 1 = molecular weight marker; 2 = *cagA*+; 3 = *m1*; 4 = *m2*; 5 = *s1a*; 6 = *s1b*; 7 = *s2* and 8 = Negative control [without DNA]).

Table 2. Prevalence of *H. pylori* genotype detected in 68 isolates

Genotype status	Patient No. (%)
cagA	
cagA +	42 (61.8)
cagA–	26 (38.2)
vacA	
m1/m2	2 (3)
s1a/m1	15 (22)
s1b/m1	4 (6)
s1a/m2	8 (12)
s1b/m2	19 (28)
s2/m1	0 (0)
s2/m2	18 (26)

Table 3. Distribution of *H. pylori* genotype in gastritis and peptic ulcer patients

Genotype status	Gastritis (n = 37) (%)	Peptic ulcer (n = 31) (%)
cagA		
cagA +	20 (54)	22 (71)
vacA		
m1/m2	0 (0)	2 (6)
s1a/m1	2 (5)	15 (48)
s1b/m1	0 (0)	4 (13)
s1a/m2	6 (16)	2 (6)
s1b/m2	16 (43)	3 (10)
s2/m1	0 (0)	0 (0)
s2/m2	17 (46)	2 (6)

only one single *vacA* genotype, and taking *vacA* s- and m-region together, three genotypes were found: *s1/m1* (28%), *s1/m2* (40%) and *s2/m2* (26%). The *s2/m1* genotype was not found in this study (Table 2).

Relationship between *H. pylori* genotyping and clinical outcomes

While estimating relationship between potentially virulent *H. pylori* strains and clinical outcomes, significant differences ($P < 0.05$) were found between isolates from gastritis and peptic ulcer cases (Table 3). The results showed a high percentage of *cagA* (70%) in peptic ulcer cases compared to gastritis cases ($P < 0.05$). The results showed a high percentage of *m2/s1* with a distribution of 22 (59%) in gastritis cases ($P < 0.05$). In case of peptic ulcer, the highest rates were among *m1/s1* with a frequency of 19 (61%) ($P < 0.05$).

DISCUSSION

The geographic distribution of distinct *H. pylori* genotypes and the prevalence of virulent bacterial genotypes in several regions, particularly in Saudi Arabia, remain unknown. This study included 68 selected patients, who were infected with *H. pylori*. In the present study, the distribution of *cagA* and *vacA* genes and their relationship to clinical outcomes were examined. The *cagA* gene was obtained with 42 isolates (61.8%). These results were in agreement with other studies conducted in Europe, Central and South America, and East Asia where a higher prevalence (67% or more) of the *cagA* genotype was reported (20). For the *vacA* genotype, and when considering a single combined genotype, the results showed that the *vacA s1* allele was predominant (68%) followed by the *vacA s2* allele (26%). These are in contrary with a study in Kuwait reported that *vacA s1* and *s2* types were detected in approximately equal numbers in biopsies obtained from patients of Middle-Eastern origin, while North Africans were predominantly infected with the *s2* type (21).

The distribution of *cagA* was 54% in gastritis and 71% in peptic ulcer cases. The relationship of the presence of *cagA* and the development of gastritis and peptic ulcer is statistically significant ($P < 0.05$), which further substantiate the role of *cagA* as a marker for increased virulence of *H. pylori*. These findings are in agreement with several previous studies (22, 23). A large number of studies have shown increased risk of gastric cancer in people with *cagA* positive *H. pylori*. However, other data have revealed that the occurrence of gastric malignancy is independent of *cag*A status. A few studies have also implicated pivotal roles of other virulence factors (*cagE*, *cagT*, *vacA*, *babA*, and *hrgA*) in the etiology of gastric cancer. Several genotypes of *H. pylori* have been reported to possess higher predictive value for the development of the severe form of the disease (24). Further study will be needed to determine the correlation between the *H. pylori* genotype and gastric cancer.

In this study, the prevalence of the *vacA* genotypes *s1/m1* was detected in 28%, *s1/m2* in 40% and *s2/m2* in 26%. No single case for *vacA s2/m1* genotype was detected in this study. This finding is in agreement with previous studies as this genotype was reported to be rare (12, 25). The most pathogenic *vacA* genotype (*vacA s1/m1*) was present in 61% of peptic ulceration cases, these are in agreement with previous studies in which association between this genotype and severe gastric outcomes were recognized. These findings support the role of *vacA s1/m1* genotype in severe clinical outcomes (13, 14, 26).

In conclusion, the genotype may be used to identify patients who are at high risk for gastroduodenal disease. *H. pylori* strains with *vacA* type s1 and combination of *s1/m1* are associated with peptic ulceration and the presence of *cagA* gene. This study suggests that further investigation is necessary to better understand the genetic diversity of this pathogen in our region.

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