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Original Article

Study of the clinical relevance of *Helicobacter pylori* virulence genes to gastric diseases among Egyptian patients

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ABSTRACT

Background and study aims: *Helicobacter pylori* infection is common in Egypt. It has been associated with gastritis, ulcers and it is a risk factor for gastric cancer. We aimed to study the correlation between the presence of *H. pylori* virulence factors and the histopathological and endoscopic findings in gastric biopsies.

Patients and methods: Gastric biopsies from thirty seven patients scheduled for diagnostic endoscopy in Cairo University hospital were included in the study. All gastric biopsies were subjected to histopathological examination and PCR assay for detection of *16S rRNA* gene to diagnose *H. pylori* infection, detection of *H. pylori* virulence factors by PCR for cagA and vacA genotypes and serological analysis of *H. pylori* (cagA, vacA, P25, and P19) IgG antibodies by immunoblot assay were done.

Results: *H. pylori* infection was detected in 23 (62.2%) cases by histopathology while 28/37 (75.7%) were positive for *H. pylori 16S rRNA* gene by PCR. By PCR seventeen samples out of 37 (45.9%) were positive for *cagA* gene and five (13.5%) for *cag* empty site gene.

Conclusion: The most common vacA genotype identified was vacA s2m2 genotype in 10 (27.02%). No statistical correlation was found between IgG antibodies against different antigens of *H. pylori* virulence factors (cagA, vacA, p25, and p19) and the degree of gastritis except for IgG antibodies against the UreA antigen.

Introduction

Helicobacter pylori infection usually starts in early years of life and tends to continue unless treated [1]. *H. pylori* is a cofactor in the development of three important upper gastrointestinal diseases; duodenal or gastric ulcers (1–10% of infected patients), gastric cancer (0.1–3%), and gastric mucosa-associated lymphoid-tissue (MALT) lymphoma (<0.01%) [2].

Several factors contribute to the degree of inflammatory response to chronic colonisation of *H. pylori* such as the virulence of the infecting strain, the host response to infection, and environmental cofactors [3].

The virulence factors of *H. pylori* and their genes serve as epidemiological markers. The *vacA* gene encodes the vacuolating toxin which induces massive vacuolisation in epithelial cells in vitro. Although all strains of *H. pylori* have a *vacA* gene, the degree of vacuolating activity differs due to sequence heterogeneity within the *vacA* gene at the 5′ end signal (s) region and the middle (m) region. Two allelic s region types have been identified, s1 and s2. Two allelic m region types have been identified, m1 and m2. The m1 type can be further subtyped as m1a and m1b [3].

The *cagA* gene is a marker for the presence of the cag pathogenicity island (cag PAI) which is an approximately 40-kb locus composed of about 27–31 genes, many of which are responsible for the synthesis of a type IV secretion system that injects the cagA oncoprotein into host cells, and the *cagA* gene (cytotoxin-associated gene) play a major role in determining the clinical outcome of *Helicobacter* infections [3,4].

We aimed to study *H. pylori* virulence factors and their possible correlation with the histological and endoscopic findings in gastric biopsies among Egyptian patients.

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Patients and methods

The present study was carried out on 37 patients who were scheduled for diagnostic endoscopy in the gastroenterology unit of Cairo University hospital. Informed consent was obtained from all cases.

Endoscopic procedure

Patient's consent to participate in this study was obtained prior to enrolment. After a fasting period of 6–8 h, patients were placed in the left lateral decubitus position, and upper endoscopy was performed with a standard forward-viewing endoscope. The patient's oropharynx was anesthetised with topical lidocaine. Intubation of the oesophagus was performed under direct vision, and then the oesophagus, stomach, and duodenum were inspected. The gastric fundus was also seen by retroverting the tip of the gastroscope.

Specimen collection and processing

After inspection of the entire gastric mucosa, multiple biopsies were taken from the gastric antrum and other areas if necessary. Part of the specimens was kept in −70 °C for further PCR technique another part was preserved in formaldehyde solution (10%) for histopathological evaluation. Serum samples were obtained from each patient.

Histopathology

For each patient; two slides were prepared from biopsy materials, one was stained by haematoxylin and eosin and the other with modified Giemsa stain; for histological evaluation of gastritis and detection of *H. pylori* in tissues.

H. pylori IgG line immunoblot assay

Serum samples were withdrawn from 37 patients and subjected to immunoblot assay using *H. pylori* line IgG immunoblot (VIROTECH) (Genzyme verotech Gmbh Lowen platz 5 D-65428 Russelsheim). The kit detects IgG antibodies for specific antigens; (cagA, vacA, ureA, p25, and p19). The test was done and interpreted according to the manufacturer's instructions.

PCR assay

DNA extraction

DNA was extracted from the gastric biopsies by using the QIAamp DNA isolation kit (Qiagen) according to the manufacturer's recommendations.

PCR analysis for 16S rRNA

PCR was performed on extracted DNA using *H. pylori 16S rRNA* specific PCR ("Hp16s"). The following cycling conditions were used: 35 cycles of 95 °C for 30 s, 60 °C for 30 s and 72° C for 30 s and an extension time of 72 °C for 5 min according to Secka et al. [5].

PCR analysis for cagA and vacA genes

PCR was performed to detect *cagA* and vacA genotypes according to Secka et al. under the following cycling conditions: 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min [5]. All amplified genes were detected by electrophoresis in 3% agarose gel with ethidium bromide. The primers used are listed in Table 1.

Statistical analysis

Statistical analysis for a possible correlation between degree of gastritis and *H. pylori* infection was conducted with Spearman's correlation test using IBM SPSS Statistics Program, version 20-2011.

Results

Histopathological evaluation of the 37 haematoxylin and eosin sections revealed two (5.4%) biopsy specimens with normal histology, 15 (40.5%) with mild gastritis, 12 (32.4%) with moderate gastritis and eight (21.6%) with severe gastritis.

The activity of inflammation in the 35 cases of gastritis, as indicated by intensity of neutrophilic infiltration, correlated mostly with degree of gastritis where 17 (48.6%) cases showed mild activity, 14 (40%) showed moderate activity and four (11.4%) showed marked activity.

Evaluation of haematoxylin and eosin and modified Giemsa stained sections revealed positivity for *H. pylori* in 23 (62.2%) cases (six cases with mild gastritis, nine with moderate gastritis and eight with severe gastritis) Fig. 1.

Spearman's correlation coefficient (r_s) was 0.4, revealing moderately significant positive correlation between the degree of gastritis and *H. pylori* infection but *p*-value was non-significant >0.01.

Atrophic changes were encountered in 16 cases (43.2% of total 37 cases), eight with mild atrophy and eight with moderate atrophy. These were evaluated by estimating the degree of glandular atrophy and cystic atrophy, irregular glandular spacing and fibrosis in the lamina propria. Intestinal metaplasia both of the complete and incomplete types were encountered in 16 cases (43.2%). Surface ulceration was encountered in five cases (13.5%) all were associated with *H. pylori* infection.

Additional histopathological findings include the presence of one case with severe gastritis and *H. pylori* infection showed reactive grade three lymphoid infiltrate that favour the diagnosis of

Table 1
Sequence of primers used for detection of *H. pylori* and *cagA* and *vacA* genotypes.

Region	Primer	Nucleotide sequence (5′-3′)	bp	Refs.
H. pylori	Hp1	CTG GAG AGA CTA AGC CCT CC	109	[6]
16 sRNA	Hp2	ATT ACT GAC GCT GAT TGT GC		
cagA	*cagA-F*	GAT AAC AGG CAA GCT TTT GAG G	349	[7]
	cagA-R	CTG CAA AAG ATT GTT TGG CAG A		
cag empty site	Luni-1	ACA TTT TGG CTA AAT AAA CGC TG	535	[7]
	R5280	GGT TGC ACG CAT TTT CCC TTA ATC		
vacA s1	Va1-F	ATG GAA ATA CAA CAA AÇA CAC	s1 at 259	[7]
vacA s2	Va1-R	CTG CTT GAA TGC GCC AAA C	s2 at 289	
vacA m1a	Va3-F	GGT CAA AAT GCG GTC ATG G	290	[7]
	Va3-R	CCA TTG GTA CCT GTA GAA AC		
vacA m2	Va4-F	GGA GCC CCA GGA AAC ATT G	352	[7]
	Va4-R	CAT AAC TAG CGC CTT GCA C		