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Original article

Helicobacter pylori Western *cagA* genotype in Egyptian patients with upper gastrointestinal disease

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ABSTRACT

Background: Infection with *Helicobacter pylori* (*H. pylori*) causes persistent gastritis that may progress to fatal gastric cancer. The cytotoxin-associated gene A protein (CagA), encoded by the *cytotoxin-associated gene A* (*cagA*) is the main virulence factor associated with more severe clinical outcomes. It is further divided into Western-type CagA and East Asian-type CagA. The East Asian-type CagA induces more cytoskeleton changes and is more likely to be associated with gastric cancer.

Aim of the study: In the current study we aimed to identify the most prevalent *H. pylori cagA* genotype among Egyptian patients suffering from dyspepsia and to examine its possible correlation with the associated clinical condition.

Patients and methods: Four biopsies were obtained from the antrum and angularis from each of 113 adult patients, who underwent upper endoscopy at the Endoscopy Unit, Theodor Bilharz Research Institute (TBRI) Hospital for the analysis of *H. pylori* by rapid urease test and detection of 16S rRNA. Nested PCR assay was used to determine *cagA* genotype.

Results: Sixty (53.1%) dyspeptic patients were found infected with *H. pylori*. Although Egypt has a high prevalence of *H. pylori* infection, low prevalence of *cagA* was detected (26.5%). Western type *cagA* is the predominant type (62.5%) while East Asian type was not detected and others (37.5%) remain uncharacterized. Western-genotype *cagA* genotype was found in 80% of patients with peptic ulcer disease and 40% of patients with gastritis.

Conclusion: Absence of the more virulent East Asian *cagA* genotype, which is the strongest risk factor for gastric carcinogenesis, may explain the very low gastric cancer rate among Egyptian population compared to other parts of the world. This finding demands further molecular studies using whole genome sequencing and more samples to determine the exact uncharacterized *cagA* genotype to identify the actual risk in developing gastroduodenal diseases in Egypt.

1. Introduction

Helicobacter pylori has been classified as a class I carcinogen by the International Agency for Research on Cancer. Infection with this pathogen causes persistent gastritis and is directly linked to the development of gastric cancer which is the fourth most common cancer and the second leading cause of cancer deaths worldwide [1,2]. Many virulence factors are involved in the pathogenicity of *H. pylori* including; urease, vacuolating cytotoxin (a product of the *vacA* gene) and the immunogenic cytotoxin-associated gene A protein (CagA), encoded by the *cytotoxin-associated gene A* (*cagA*). While the *vacA* gene is present in all strains of *H. pylori*, *cagA* is not. The *cagA* is a virulence gene located in the *cagA* pathogenicity island *(cag PAI)* of the bacterial genome and is frequently associated with more severe clinical outcomes. The *cagA* encodes proteins that increase the virulence potencies of strains; by increasing host-cell cytokine production and altering protein tyrosine phosphorylation [3].

The CagA protein is further divided into Western-type CagA and East Asian-type CagA, by the repeat sequence Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs at its N terminus [4]. Four different CagA EPIYA

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motifs have been identified; EPIYA-A, -B, -C, and -D have been defined based on the amino acid sequences surrounding the EPIYA residue. CagA proteins nearly always possess an EPIYA-A and an EPIYA-B, followed by various numbers of EPIYA-C repeats in Western-type or EPIYA-D motifs in East Asian type strains. It has been suggested that the considerable variation in number of repeating EPIYA-C or -D motifs determines the biological activity of CagA. When CagA is injected from the bacteria into gastric epithelial cells, it undergoes tyrosine phosphorylation and binds to Src homology 2 domain-containing protein-tyrosine phosphatase (SHP-2). SHP-2 is known to play an important positive role in mitogenic signal transduction. In addition, SHP-2 is actively involved in the regulation of spreading, migration and adhesion of cells. So, the deregulation of SHP-2 by translocated CagA may induce abnormal proliferation and movement of gastric epithelial cells [5]. The affinity of the East Asian-type CagA to SHP-2 is significantly higher than that of the Western-type CagA. Thus, East Asian-type CagA induces more cytoskeleton changes and is more likely to be associated with gastric cancer [4].

Therefore, extensive genotyping of the *cagA* gene has been carried out in many countries using *H. pylori* strains isolated from patients with gastric diseases [3,6,7].

Since the East Asian CagA *H. pylori* strains are associated with higher gastric pathogenicity, we aimed to identify the most prevalent *H. pylori cagA* genotype among Egyptian patients suffering from dyspepsia and to examine its possible correlation with associated clinical condition. To the best of our knowledge this is the first study from Egypt that investigates *cagA* genotyping and its possible outcome clinically.

2. Subjects and methods

2.1. Patients and specimens

A total number of 113 adult patients, who underwent upper endoscopy with various dyspepsia symptoms (abdominal or epigastric pain, vomiting and/or heartburn) at the Endoscopy Unit, Theodor Bilharz Research Institute (TBRI) Hospital from March 2013 to February 2016, were enrolled in this cross-sectional study. None of them received non-steroidal anti-inflammatory drugs, antibiotics, H2 receptors antagonists or proton pump inhibitors in the past four weeks prior to the study. Thorough endoscopic examination of the oesophagus, stomach and duodenum was done together, as well as clinical assessment of the patient condition (gastritis, peptic ulceration, normal endoscopy and other findings). From each patient, four biopsies were obtained from the antrum and angularis in 2 tubes. One tube was tested for rapid urease test (Bussero, Milan, Italy) and the other tube was stored in sterile physiological saline in sterile Eppendorf tubes and kept at −70 °C in sterile physiological saline until processed as panel for DNA extraction and PCR assays. The protocol was approved by TBRI institutional review board (FWA00010609) and all patients provided a written informed consent.

2.2. DNA extraction from gastric biopsy specimens

Genomic DNA was extracted from gastric biopsy specimens using (QIAamp DNA Mini Kit (50) 51304 from QIAGEN, USA), catalogue number #51304 following manufacturer guidelines.

2.3. PCR analysis

The PCR analysis for *16S rRNA* was done to confirm presence of *H. pylori* according to Chisholm et al. (2001) [8]. To detect *cagA* genotype, nested PCR assay was performed as described by Hirai et al. (2009) [9,10], using genotype-specific primers and two rounds of PCR. The first round was performed using a common forward primer (F1) and either of the two reverse primers (R1 or R2) (Table 1). The PCR cycling conditions for the first round were as follows: 95 °C for 10 min, then 40 cycles at 94 °C for 15 s, 55 °C for 30 s and 68 °C for 30 s. The second round was performed using 10 μl of the PCR products obtained in the first round as the template. In the second round, primers specific to these two types were used in separate reactions. The cycling conditions of the second round of PCR were as follows: 94 °C for 2 min, then 30 cycles at 98 °C for 10 s and 63 °C for 30 s. PCR amplifications were carried out Bio Rad T100 thermal cycler, USA. Each PCR product was separated on 2% agarose gel with ethidium bromide and 100 bp ladder used as DNA molecular weight standard.

2.4. Statistical analysis

Results are expressed as number (%). Comparison between categorial data [number (percent)] was performed Chi square test. Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant.

3. Results

Sixty (60) out of 113 (53.1%) dyspeptic patients were found infected with *H. pylori* by positive rapid urease test and confirmed by *16S rRNA* PCR. Among the studied 60 *H. pylori* infected patients, 40 (66.7%) patients were males and 20 (33.3%) were females. Ages of the patients ranged between 17 and 76 years (49.93 ± 14.28 years). Eighty-five percent (85%) (51/60) of the patients were from urban regions while 15% (9/60) were from rural areas. Half of the *H. pylori* infected patients (31/60, 51.7%) were above 51 years, 46.7% (28/60) were between 21 and 50 years, while only one patient (1.7%) was below 20 years.

Upper gastrointestinal endoscopy revealed gastritis in 27(45%) and peptic ulcer disease (PUD) in 10 (16.7%). Other endoscopic

Table 1
Oligonucleotide primers used for PCR analysis.

Target Genes	Primer	Primer Sequence	Amplicon	Reference
16S rRNA	F	5′-CTG GAG AGA CTA AGC CCT CC-3′	110 bp	[8]
	R	5′-ATT ACT GAC GCT GAT TGT GC-3′		
cagA	F1	5′-GGA ACC CTA GTC AGT AAT GGG TT-3′	550 bp	[910]
	F2	5′-CCA ATA ACA ATA ATA ATG GAC TCAA-3′		
	R1	5′-GCT TTA GCT TCT GAT ACC GCT TGA-3′		[910]
	R2	5′-AAT TCT TGT TCC CTT GAA AGC CC-3′		
cagA-Western	F	5′-AGG CAT GAT AAA GTT GAT GAT-3′	92 bp	[5]
	R	5′-AAA GGT CCG CCG AGA TCA T-3′		
cagA-East-Asian	F	5′-AAA GGA GTG GGC GGT TTC A-3′		
	R	5′-CCT GCT TGA TTT GCC TCA TCA-3′		