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Original article

Detection of *Helicobacter pylori vacA*, *cagA* and *iceA1* virulence genes associated with gastric diseases in Egyptian patients

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ABSTRACT

Background: *Helicobacter pylori* (*H. pylori*) virulence markers would be useful to predict peptic ulcer disease (PUD) or gastric cancer.
Aim: In Egypt, since inadequate data are present regarding *H. pylori* virulence–related genes in different age group patients with gastro-duodenal diseases, it becomes crucial to study the clinical status of *cagA*, *vacA* and *iceA*1 genotypes of *H. pylori* strains recovered from patients with dyspepsia.
Subjects and methods: The study included 113 dyspeptic patients who were exposed to upper gastrointestinal endoscopic examination. Four antral biopsies were obtained from each patient for the analysis of *H. pylori* infection by rapid urease test and detection of *16S rRNA*.
Results: Sixty (53.1%) patients were confirmed to be infected with *H. pylori*. Upon endoscopy, gastritis was revealed in 27 patients (45%) and10 patients (16.7%) had PUD. Of the 60 *H. pylori* strains, 39 (65%) had at least one virulence gene. Six different genotypic forms were recognized; *vacA* (9/60), *iceA1* (1/60), *vacA/cagA* (7/60), *vacA/iceA1* (13/60), *vacA/cagA/iceA1* (8/60) only one of *cagA/iceA type* and we could not detect *cagA*. The overall *vacA*, *iceA1*and *cagA* genes identified were 61.6%, 38.8%, 26.6% respectively, by PCR-based molecular testing. The *vacA* gene status was highly significant related to gastritis patient ($P \leq 0.036$). The *vacA s1m1* and *s2m2* alleles were significantly found in 50% of *H. pylori* infected patients with PUD and with gastritis 57.1% respectively ($P \leq 0.01$).
Conclusion: In conclusion, the main genotype combinations in the studied Egyptian patients were; *vacAs2m2/iceA1*, *vacAs1m1/cagA,* mostly associated with gastritis, and *vacAs1/cagA/icA*, mainly in PUD. The less virulent *(s2, s2m2) H. pylori* genotypes were found in patients aged over 43 years.

1. Introduction

Helicobacter pylori (*H. pylori*) colonizes the gastric mucosa of the human stomach and establishes long-term infection. Dyspepsia is the most common gastrointestinal disorder, and is the most common indication for gastric endoscopy [1]. Infection with *H. pylori* have been proven to be highly associated with gastritis, peptic ulcer disease (PUD) and adenocarcinoma [2,3]. Since 1994, The World Health Organization (WHO) classified *H. pylori* as Class I carcinogen because of its causal relationship to gastric carcinoma [4–6]. The extent and the severity of these associations depend on several elements, such as bacterial virulence factors, age of the host, genetic susceptibility, immune response and environmental factors [7].

H. pylori has a number of virulence factors that play a role in its pathogenicity and influence its colonization and disease severity. CagA, encoded by *cagA* gene is a part and a marker of cagA Pathogenicity Island (PAI). *CagA*-positive *H. pylori* strains are associated with severe inflammation and increased risk of ulcers and cancer in humans. The presence of *cagA* usually coincide with the

Abbreviations: H. pylori, *Helicobacter pylori*; PUD, peptic ulcer disease; WHO, World Health Organization; PAI, Pathogenicity Island; TBRI, Theodor Bilharz Research Institute; DNA, deoxy-ribonucleic acid; PCR, polymerase chain reaction; 16S rRNA, 16 S ribosomal ribonucleic acid; dNTPs, deoxynucleotide triphosphates; GERD, gastroesophageal reflux disease; SPSS, Statistical Package for Social Sciences; Bp, base pair; Lab, laboratory.

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presence of other virulence factors, including *vacA* [8,9]. VacA is an *H. pylori* toxin with multiple cellular effects in different host cell types. Virtually all *H. pylori* strains produce VacA. However, there is significant variation among strains in their capacity to induce cell vacuolization. This variation is attributed to the genetic structural diversity of the *vacA* gene that can assume different polymorphic rearrangements [10]. The initial studies on *vacA* detected two main polymorphic regions; the signal (s)- and the middle (m)-regions. The s- region assumes two forms *s1* or *s2* allele and the m-region encoded the *vacA m1* or *m2* allele. The *vacA* type *s1* strains appear to be more active than s2 strains and are found more frequently in ulcer disease. The *vacA m1* type strains are associated with greater gastric epithelial damage than *m2* strains. The combination of s- and m-region allelic types determines the production of the cytotoxin and is associated with pathogenicity of *H. pylori* strains. *vacA s1/m1* strains produce a large amount of toxin, *s1/m2* strains produce moderate amounts and *s2/m2* strains produce very little or no toxin [11–13].

The *iceA* gene has two main allelic variants; *iceA1* and *iceA2*. The expression of iceA1 is up-regulated on contact between *H. pylori* and human epithelial cells, and may be associated with peptic ulcer disease [14]. However, several studies were not able to explain the role of *iceA* and its correlation with clinical outcomes in other populations; therefore the mechanism of how *iceA* induce PUD remains unclear. Such contradicted results between the *iceA* genotype and clinical consequences could be explicated by the genetic diversity or differences in the geographic region, which were previously reported for other virulence factors [15].

In Egypt, since inadequate data regarding *H. pylori* virulence – related genes in different age group patients with gastroduodenal diseases (gastritis and peptic ulcer disease), it becomes crucial to study the clinical status of *cagA*, *vacA* and *iceA*1 genotypes of *H. pylori* strains recovered from patients with dyspepsia.

2. Subjects and methods

2.1. Patients and clinical specimens

A total number of 113 adult patients undergoing 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 January 2015 were enrolled in this study. Patients who had received non-steroidal anti-inflammatory drugs, as well as antibiotics, H2 receptors antagonists or proton pump inhibitors four weeks prior to the study were excluded. During upper gastrointestinal endoscopy (Olympus X Q40) for examination of the oesophagus, stomach and duodenum, all abnormalities (gastritis, ulceration, erosion and others) were recorded. Four antral biopsies were obtained from each patient. One biopsy was tested for rapid urease test that was performed using rapid urease liquid test kit (Bussero, Milan, Italy), and the other three gastric biopsies were stored in sterile physiological saline in sterile Eppendorf tubes and kept at −70 °C until processed as panel for Deoxyribonucleic acid (DNA) extraction, were used directly for Polymerase chain reaction (PCR) assays. A patient was considered to be infected with *H. pylori* when he had positive rapid urease test and confirmed by detection of *16S rRNA* in gastric biopsy specimens. The protocol was approved by Theodor Bilharz Research Institute (TBRI) institutional review board (FWA00010609) and all patients provided a written informed consent.

2.2. DNA extraction from gastric biopsy specimens and PCR assays

Genomic DNA was extracted from gastric biopsy specimens using (QIAamp DNA Mini Kit (50) 51304 from QIAGEN, USA), catalogue number #51304 following manufacturer guidelines. PCR for *16S* rRNA, *cagA, vacA* and *iceA1* gens were performed in a volume of 50ul with approximately 5 μg of extracted DNA, 200 μM (each) dNTPs, 25 pmol for each primer (Table 1), 1.5 μM Magnesium Chloride and 1unit of *Taq* polymerase (Gotaq Flexi DNA, M8305, Promega, Inc, USA). The reaction was done in PTC-100™ thermal cycler (MJ Research, USA), programmed as follows: denaturing at 95 °C for 5 min, followed by 37 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min, and final extension at 72 °C for 5 min. [13,16,17].

Each PCR product was separated on 2% agarose gel with ethidium bromide, and 50 bp ladder used as DNA molecular weight standard. In each PCR assay, a negative control (lacking DNA) was include

3. Results

Out of 113 patients included in the study, 60 (53.1%) were confirmed to be infected with *H. pylori*. They presented mostly with dyspepsia 34 (56.7%) followed by vomiting 16 (26.7%) and then heart burn 10 (16.7%).

Upper gastrointestinal endoscopy of the 60 *H. pylori* infected patients revealed gastritis in 27 (45%), whereas 10 (16.7%) had peptic ulcer disease and 4 (6.7%) had normal gastric mucosa. Other endoscopic findings as gastric prolapse, gastroesophageal reflux disease (GERD), oesophageal varices, esophagitis and hiatus hernia were detected in 19 (31.7%) (Table 2).

DNA extracted from gastric antral biopsy specimens was used directly for detection of *H. pylori 16S rRNA*. The *vacA, cagA* and *iceA1* genotypes of *H. pylori* were analysed for the 60 *H. pylori* strains by PCR assays.

3.1. Association between virulence genes and clinical outcomes in 60 H. pylori strains

Of the 60 *H. pylori* strains tested, 39 (65%) had at least one virulence gene and 21 (35%) did not show any virulence gene (Table 2).

In the current study, 48.3% (29/60 cases) of *H. pylori* positive specimens had two or three of the virulence genes examined in this study as evidenced by PCR-based molecular testing.

Six different genotypic combinations can be recognized. Considering the presence of only one genotype marker; *vacA* (15%, 9/60) and *iceA1* (1.7%, 1/60) were confirmed. We could not detect *cagA* as a single genotype.

Other genotype combinations were detected; *vacA+/cagA+*(11.7%,7/60), *vacA+/iceA1+*(21.7%,13/60), *vacA+/cagA+/iceA1+*(13.3%, 8/60) and only one *H. pylori* strain of *cagA+/iceA +* existed in a patient with endoscopic findings other than gastritis and PUD.

Among PUD patients (n = 10); 4 (40%) had *vacA+/cagA+*; 3/4 had *vacA s1m1* genotype and one (1/4) had *s2m2*. The *vacAs1m2+/iceA1 +* and *vacA s1m1+/cagA+/iceA1 +* genotype combination were detected in one (10%) patient each. While for gastritis patients (n = 27); 5 (18.5%) were of genotype *vacAs2m2+/iceA1+*, 5 (18.5%) had *vacA*s1+/*cagA+/iceA1+*; 3 were of *vacA s1m1* genotype and 2 were of *vacA s1m2* genotype.

In the present study, not all the patients in whom gastritis (12/27) and PUD (2/10) were diagnosed were infected with virulent genotype *H. pylori.*

3.2. vacA Genotypes Status

The *vacA* gene was detected in 37 (61.6%) of the 60 *H. pylori* studied strains. The *vacA* s-region and m-region were determined in all 37 *vacA* positive strains. *vacA* (*s1*) allele was found in 17/37

Table 1
PCR primers used for amplification of *16S rRNA*, *cagA*, *vacA* and *iceA1* sequences assays.

Target genes		Primers sequences (5′-3′)	Amplicon bp	References
16S rRNA		5′CTG GAG AGA CTA AGC CCT CC-3′ 5′ATT ACT GAC GCT GAT TGT GC-3′	110	[16]
cagA		5′AAT ACA CCA ACG CCT CCA-3′ 5′TTG TTG CCG CTT TTG CTC TC-3′	400	[17]
vacA	*vacA (s1/s2)*	5′ATG GAA ATA CAA CAA ACA CAC-3′ 5′CTG CTT GAA TGC GCC AAA C-3′	259/286	[17]
	vacA (m1/m2)	5′CAA TCT GTC CAA TCA AGC GAG-3′ 5′GCG TCT AAA TAA TTC CAA GG-3′	570/642,	[17]
iceA1		5′ GTG TTT TTA ACC AAA GTA TC-3′ 5′ CTA TAG CCA STY TCT TTG CA-3′	246 bp	[13]

Table 2
Association between virulence genes and clinical outcomes in 60 *H. pylori* strains.

Virulence genes status	*vacA* Genotypes	Endoscopic findings			
Positive strains (n = 39)		Gastritis (n = 27)	PUD[a] (n = 10)	Normal Mucosa (n = 4)	Others[b] (n = 19)
vacA+ (n = 9)	*s1m1*(n = 1)	0	0	0	1
	s1m2(n = 1)	1	0	0	0
	*s2*m (n = 1)	0	0	1	0
	s2m2(n = 6)	3	1	0	2
vacA+/cagA+ (n = 7)	*s1m1*(n = 5)	0	3	0	2
	s1m2(n = 1)	0	0	0	1
	s2m1(n = 0)	0	0	0	0
	s2m2(n = 1)	0	1	0	0
vacA+/iceA1+ (n = 13)	*s1m1*(n = 0)	0	0	0	0
	s1m2(n = 1)	0	1	0	0
	s2m1(n = 0)	0	0	0	0
	s2m2(n = 12)	5	1	0	6
vacA+/cagA+/iceA1+ (n = 8)	*s1m1*(n = 4)	3	1	0	0
	s1m2(n = 4)	2	0	0	2
	s2m1(n = 0)	0	0	0	0
	s2m2(n = 0)	0	0	0	0
cagA+/iceA1+ (n = 1)		0	0	0	1
IceA1+ (n = 1)		1	0	0	0
CagA+ (n = 0)		0	0	0	0
Negative Strains (n = 21)					
vacA-/cagA-/iceA1- (n = 21)		12	2	3	4

Data are expressed as numbers.
[a] PUD: Peptic ulcer disease.
[b] Other endoscopic findings which include; gastric prolapse, GERD, oesophageal varices, oesophagitis and hiatus hernia.

(45.9%), *vacA* (*s2*) allele was found in 20/37 (54%) (Fig. 1), *vacA* (*m1*) allele was found in 11/37 (29.7%) whereas *vacA* (*m2*) allele was found in 26/37 (70.2%) (Fig. 2). allele combination: four *vacA* genotypes were identified, the most dominant one was *s2m2* allele combination 19/37 (51.3%), followed by *s1m1* 10/37 (27.0%), *s1m2* 7/37 (18.9%) and *s2m1* genotype was found in just one strain (Table 2).

Regarding the endoscopy aspects of the mucosa of *H. pylori* infected patients, 8/10 patients with PUD (80%) and 14/27 (51.8%) with gastritis were positive for *vacA* genotype. The more frequent *vacA* allele among patients with gastritis was *m2* (40%) followed by *s2* (29.6%) and *s1* (22.2%), while for patients with PUD; (55.5%), (50%) (40%) (44.4%) and (40%)(33.3%) were of *vacA s1, m1* and *m2* alleles respectively. So *vacA s1m1* and *s2m2* were significantly found in 50% (4/8) *H. pylori* infected patients with gastritis 57.1% (8/14) ($p < 0.01$) respectively (Table 3).

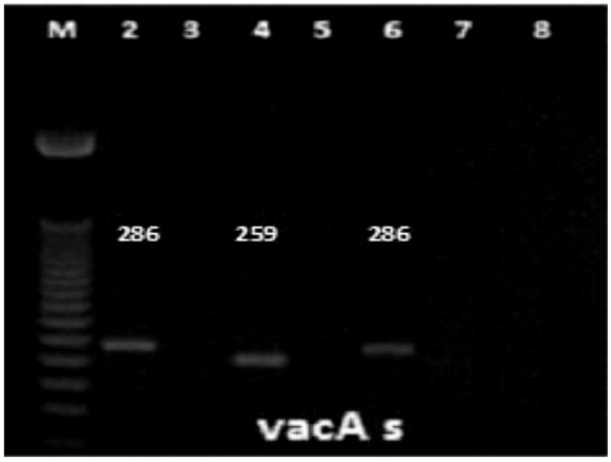

Fig. 1. Agarose gel electrophoresis PCR products amplified of *H. pylori vacA s-* region. Lane M: Molecular weight marker (50 bp). Lanes 2,6: *s2*-region (286 bp). Lane 4: *s1*-region (259 bp). Lane 8: Negative control.

3.3. cagA Genotype Status

The *cagA* gene was detected in 16 (26.6%) of the 60 *H. pylori* strains (Table 2). Upon endoscopy, 5/10 (50%) and 5/27 (18.5%) of

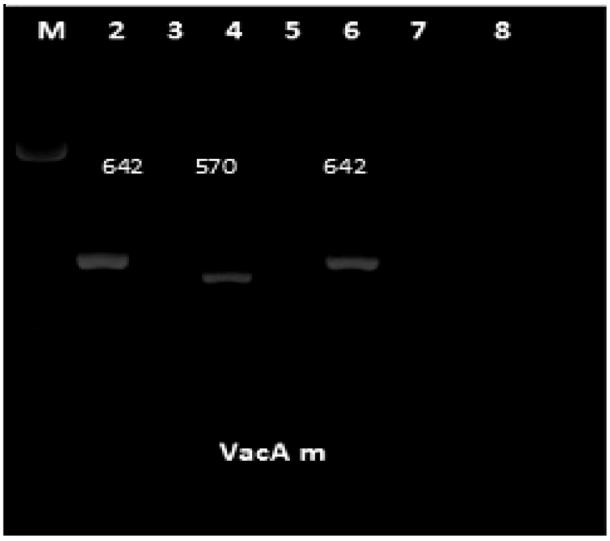

Fig. 2. Agarose gel electrophoresis of PCR products of *H .pylori vacA m*-region. Lane M: Molecular weight marker (50 bp). Lanes 2, 6: *m2*-region (642 bp). Lane 4: *m1*-region (570 bp). Lane 8: Negative control.

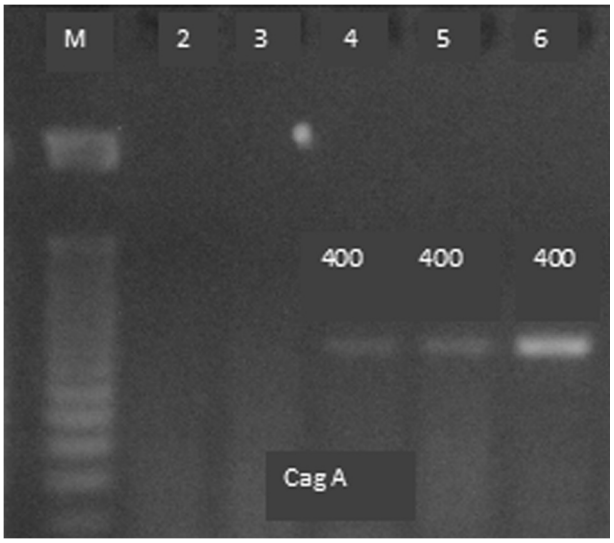

Fig. 3. Agarose gel electrophoresis for PCR products of *H .pylori* of *cagA* status. Lane M: Molecular weight marker (50 bp). Lane 2: Negative control. Lanes 4–6: Positive *cagA* gene (400 bp).

H. pylori infected patients with PUD and gastritis respectively were associated with *cagA* genotype (Table 3, Fig. 3).

3.4. iceA1 Genotype Status

The *iceA1* gene was detected in 23 (38.3%) of the 60 *H. pylori* strains (Table 2). Eleven out of 27 (40.7%) *H. pylori* infected patients with gastritis and 3 out of 10 PUD patients were associated with *iceA1* genotype (Table 3, Fig. 4).

A relation was found between *vacA* followed by *iceA1* status and all clinical abnormalities detected by upper endoscopy, with variable ranges. It was highly significant (p value $\leq$ 0.036) compared to *cagA* status in cases of gastritis. However, *vacA* and *cagA* genes were of borderline insignificance relation in case of PUD (P value $\leq$ 0.079) compared with *iceA1* gene.

4. Association between Age of Patients and *H. pylori* Genotypes

From the studied 60 *H. pylori* strains, the patients' ages ranged between 17 and 76 years (49.93 ± 14.28 years). *H. pylori* infection increases with age, and can be found more in the population aged $\geq$ 43 years (43/60, 71.7%), on the other hand, 28.3% (17/60) were < 43 years. Genotypes *S2* and *S2m2* were more frequently found in patients aged 43 years or more (P value $\leq$ 0.025 and 0.039, respectively), while the association between genotype *S1* genotype and the latter group of age was insignificance (Table 4).

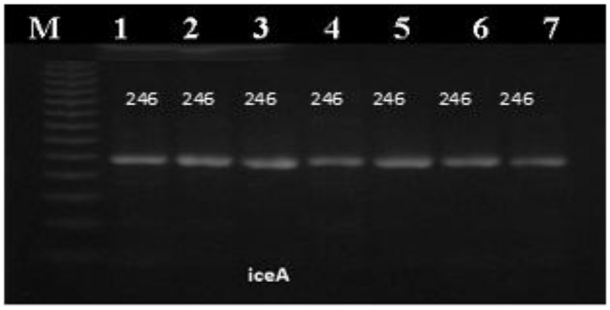

Fig. 4. Agarose gel electrophoresis of PCR products of *H. pylori iceA* positive gene (246 bp) from gastric biopsy on agarose gel. Lane M: molecular weight marker (ladder 50 bp). Lanes (1–7): *iceA* gene. Lane N: negative control.

5. Statistical analysis

Results are expressed as number (%). Comparison between categorical data Statistical Package for Social Sciences (SPSS) com-

Table 3
Total number and percentage of *H. pylori* strains virulence genes *vacA*, *cagA* and *iceA1*.

Virulence Genes	Endoscopic findings of 60 *H. pylori* infected patients				Total
	PUD[a] (N = 10)	Gastritis (N = 27)	Normal Mucosa (N = 4)	Others[b] (N = 19)	
vacA+ (n = 37)	8 (80.0)	14 (51.8)*	1 (25.0)	14 (73.6)	37 (61.7)
s1m1 (n = 10)	4 (50.0)**	3 (21.4)	0 (0.0)	3 (21.4)	
s1m2 (n = 7)	1 (12.25)	3 (21.4)	0 (0.0)	3 (21.4)	
s2m1 (n = 1)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	
s2m2 (n = 19)	3 (37.5)	8 (57.1)**	0 (0.0)	8 (42.1)	
vacA - (n = 23)	2 (20.0)	13 (48.1)	3 (75.0)	5 (34.8)	23 (38.3)
cagA+ (n = 16)	5 (50.0)	5 (18.5)	0 (0.0)	6 (31.5)	16 (26.7)
cagA- (n = 44)	5 (50.0)	22 (81.5)	4 (100)	13 (73.9)	44 (73.3)
iceA1+ (n = 23)	3 (30.0)	11 (40.7)	0 (0.0)	9 (47.3)	23 (38.3)
iceA1-(n = 37)	7 (70.0)	16 (59.3)	4 (100.0)	10 (60.9)	37 (61.7)

Data are expressed as number (%).
[a] PUD: Peptic Ulcer Disease.
[b] Other endoscopic findings which include; gastric prolapse, GERD, oesophageal varices, oesophagitis and hiatus hernia.
* P-value $\leq$ 0.036.
** P-value $\leq$ 0.01.

puter program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant and < 0.01 was considered highly significant.

6. Discussion

Our study investigated the relation of the *cagA*, *vacA* and *iceA1* virulence genes in *H. pylori* strains, obtained from gastric biopsy specimens in a group of dyspeptic patients admitted to the Endoscope Unit at TBRI Hospital, and to examine the association between such genes with the clinical outcomes evidenced by endoscopic findings.

In the current study, four antral biopsy specimens were obtained from each patient with dyspepsia for rapid urease test and positive results were confirmed by detection of *16S rRNA* gene by PCR assay. The prevalence rate of *H. pylori* (53.1%) in the studied patients was comparable with results recorded by Goh [18] from Malaysia, where the prevalence of *H. pylori* was 49.0% and in other neighbouring countries, in Kuwait and Saudi Arabia, where the prevalence of *H. pylori* infection was 49.7% and 49.8% respectively [19,20]. However, our figure was lower than previous Egyptian studies by Zaki et al. [21]; Ali and Borei [22]; and Abu-Zekry et al. [23] where *H. pylori* infection was recorded in 60.9%, 62% and 70%; respectively by culturing gastric biopsy specimens, using C-urea breath test and *H. pylori* antigen in stool respectively.

Such variation in *H. pylori* infection prevalence rates from country to country and even among population groups within the same country could be due to diverse contributing factors including socioeconomic status, geographical or living conditions and location of each population. The developing countries have much higher infection rates around 20–90% probably than developed countries where rates are estimated to be around 10–60% [24,25].

H. pylori colonizes the stomach and elicits a gastric mucosal inflammatory response termed "gastritis" in both humans and experimentally infected animals. Such condition can persist for many decades in the absence of antimicrobial treatment. Longitudinal studies indicate that gastritis is one of the first detectable changes in a stepwise pathway of histological abnormalities that can ultimately culminate in gastric cancer [26,27].

Upon upper gastrointestinal endoscopy of the studied *H. pylori* infected patients, the most common finding was gastritis (45%) followed by peptic ulcer (16.6%). Our results were in agreement with Ngoyi et al. [28]. Peptic ulcer was detected in 15.9% of cases, and in a study from Pakistan, Rasheed et al. [29] reported that gastritis and peptic ulcer were 65.2% and 67.3%; respectively.

H. pylori pathogenesis and disease outcomes are mediated by a complex interplay between bacterial virulence factors, host and environmental factors [30].

DNA extracted from the studied gastric antral biopsy specimens was used directly for detecting *H. pylori vacA, cagA* and *iceA* variants genotypes, 48.3% of *H. pylori* positive specimens had two or three of the virulence genes examined in this study. This suggests that the majority of *H. pylori* strains examined had virulence potential as evidenced by PCR-based molecular testing.

Some authors have declared that there could be a relationship between *H. pylori* strain genotype and clinical outcome, enabling the prediction of the latter [31]. Strains of *H. pylori* have been previously classified as type I (*cagA+*, *vacA+*) and type II (*cagA-*, *vacA-*, according to the presence/absence of the genes). Type I strains are considered to be more virulent than type II strains [32]. Other authors have reported that triple-positive strains *vacA+/cagA+/iceA1+* were linked to severe diseases [13].

According to our results, the *cagA+/vacA+* genotype was mostly from patients with PUD (4/7) and particularly of *vacAs1m1* (3/4) genotype, which was consistent with a previous study [33] which confirms the association between such *vacA* genotype and severe gastric outcomes. Our findings also coincide with a recent report from Venezuela in which genotype *cagA* was not observed as a single genotype, but it was commonly associated with *vacA s1 m1*and in PUD and gastritis [34]. The *vacAs1* and *m1* genotypes were considered to be indicators of the *cagA*-positive status in *H. pylori* strains of these patients with peptic ulcer [35]. Nevertheless, Gonzalez- Vazquez [36] found that type I *H. pylori* strains were more isolated from patients with gastritis rather than those with duodenal ulcer disease.

Most of the detected *vacA+/icA1 +* genotype (12/13) were of the less virulent *vacA s2m2* genotype and were mainly isolated from gastritis patients. A meta-analysis study confirms the relationship between the *iceA* allelic type and clinical outcomes [37]. Several studies suggest an association of the *iceA1*variants and PUD [38,39]. However, this association varies among populations in Brazil, for example, where *iceA1* allele is associated with gastritis [40].

The triple-positive strains, *vacA s1+/cagA+/iceA1+* (5/8), were detected from the studied gastritis patients. On the other hand Van Doorn [13] reported that strains that are typed as *vacA s1/cagA/iceA1* can be considered as the most pathogenic and are found predominantly in patients with ulcer disease.

According to our findings, we could not find a significant relationship between genotype combinations and clinical outcomes. However, sample size could be increased to establish such relation.

The geographic origin may play a role in the discrepancy between several reports and the results from the present study. The overall *vacA* gene was detected in 61.6% of the studied *H. pylori* strains, being the main virulent gene found in most of the studied strains as in other previous reports [41,42]. The variability of *vacA* gene has been previously associated with geographic regions that include the Middle East where the frequencies of the *vac A s* genotype differed significantly between its southern part (Kuwait, Jordan, Saudi Arabia and Israel) and the northern part (Turkey, Iran and Iraq). The higher detection rate of the *vacA s1* genotype was revealed from South Asian countries, such as India and Bangladesh, to East Europe throughout the northern part of the Middle East [43]. In our study, *vacA s1* and *m1* alleles detection rate was comparable with Van Doorn et al. [44], who reported that the prevalence of the *vacA s1* and *m1* genotypes in strains from Egypt was 42.9% and 14.3%, respectively. Such finding suggested that the population which lived in the southern part of the Middle Eastern population near African Arabs might correlate culturally with African countries and not the north side of the Middle Eastern population. Despite of the high levels of *H. pylori* infection in the Middle East [45,46], there was a widespread prevalence of weakly cytotoxic strains which may be the reason for the low frequency of gastric cancer[47]. Our results revealed a higher detection rate of *vacA s2m2* genotype that is considered to be a less virulent strain than *vacA s1m1* which is supposed to be highly active and can damage cells in a more acute manner [48]. Also, we found the mosaic genotype of *vacA s1m2* and the least prevalent in this work, *vacA s2m1*, to be consistent with the results of children cases in Iran [49].

Among *H. pylori* strains that produce *cagA*, there is variation in its level of production. The epidemiological prevalence of *cagA*-positive *H. pylori* infection in western countries is nearly 60% [50], and the prevalence is about 90% in Asian countries [51]. The overall *cagA* gene was detected in 26.6% of the studied *H. pylori* strains comparable to previous Egyptian study by Diab et al. [52], but it was lower than other Egyptian studies (66.6%, 62.2%) by El-Fakhry et al. [53], and Zaki, [21] respectively. In Europe Saribasak et al. [54], in Saudi Arabia Kadi [15] and East Asia Saribasak et al. [54], *H. pylori* strains harboured *cagA* genes (70%, 81.8% and 90% respectively) which were higher than what occurred in the current study.

Table 4
Association between Age of Patients and *H. pylori* Genotypes.

Age Group	*H. pylori* Genotypes									
	s1	*s2*	*m1*	*m2*	*s1m1*	*s1m2*	*s2m1*	*s2m2*	*cagA*	*iceA1*
Age < 43 Years No. (17)	6	5	5	6	5	1	0	5	9	7
Age ≥ 43 Years No. (43)	11*	15**	6	20	5	6	1	14***	7	16

Data are expressed as number.
* P–value ≤ 0.225.
** P-value ≤ 0.025.
*** P-value ≤ 0.039.

A recent study showed that strains producing high levels of CagA are linked to an increased risk of premalignant lesions compared to strains producing lower levels of CagA [55]. Kao et al. [30] reported that the virulence of individual *H. pylori* isolates has been measured by their ability to produce CagA. Our positivity rates of 55.5% and 18.5% of *H. pylori* infected patients with PUD and gastritis respectively were associated with *cagA* genotype. Such result was comparable with previous studies [56,57]. The correlation between positive *cagA* status and severe clinical outcomes was reported in some other studies [15,49].

Although previous studies reported by Van Doorn [13]; and Kadi [15] detected that *iceA1* strains are much more prevalent among patients with PUD, yet we detected *iceA1* genotype more frequently in gastritis as compared with PUD patients.

In a study done by Momenah and Tayeb, [58], they found that 12.6% of the positive *H. pylori* cases were *iceA1* positive. As reported by Ashour et al. [59]; and Ben Mansour et al. [60] *iceA1* expression is associated with a higher activity of the gastric inflammation, a condition that increases the risk for developing ulcer disease and gastric carcinoma.

The current study showed significant association of more virulent variants of *H. pylori (s1 and s1m2)* strains in the group of older patients. Such finding coincides with [42]. In Portugal and Tunisia, *H. pylori* virulent strains have been detected in adult patients more frequently than children [60].

7. Conclusion

Characterization of *H. pylori* virulent markers could permit identification of high-risk patients, as the prevalence rate of *H. pylori* infection among dyspeptic Egyptian patients is high. The main genotype combinations in the studied Egyptian patients were; *vacAs2m2/iceA1, vacAs1m1/cagA*, mostly associated with gastritis, and *vacAs1/cagA/icA*, mainly in PUD. The less virulent *(s2, s2m2) H. pylori* genotypes were found in patients aged over 43 years. Eventually, multiple strain colonization should be considered when planning for prophylactic and therapeutic strategies to prevent the development of sever diseases.

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References

[1] Olokoba AB, Gashau W, Bwala S, Adamu A, Salawu FK. *Helicobacter pylori* infection in Nigerians with dyspepsia. Ghana Med J 2013;47:79–81.
[2] Marshall B, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;1:1311–5.
[3] Blaser M. Who are we? Indigenous microbes and the ecology of human diseases. EMBO Rep 2006;7:956–60.
[4] International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Schistosomes, Liver Flukes and *Helicobacter Pylori*, vol. 61. International Agency for Research on Cancer; 1994.
[5] Treiber G, Malfertheiner P and Klotz U, Treatment and dosing of Helicobacter pylori infection: when pharmacology meets clinic 2007; 329–350.
[6] World Health Organization, Cancer Fact Sheet; 2007. http://www.who.int/media centre/fact sheets/fs297/en.
[7] Markovska R, Boyanova L, Yordanov D, Gergova G, Mitov I. *Helicobacter pylori* oipA genetic diversity and its associations with both disease and *cagA, vacA s, m*, and *i* alleles among Bulgarian patients. Diag Microbiol Infect Dis 2011;71:335–40.
[8] Yamaoka Y. Mechanisms of disease: *H. pylori* virulence factors. Nat Rev Gastroenterol Hepatol 2010;7:629–41.
[9] Kim SS, Ruiz VE, Carroll JD, Moss SF. *H. pylori* in the pathogenesis of gastric cancer and gastric lymphoma. Cancer Lett 2011;305:228–38.
[10] Ferreira RM, Machado JC, Figueiredo C. linical relevance of Helicobacter pylori vacA and cagA genotypes in gastric carcinoma. Best Pract Res Clin Haematol 2014;28:1003–15.
[11] Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J Biol Chem 1995;270:17771–7.
[12] Cover TL. The vacuolatingcytotoxin of *H. pylori*. Mol Microbiol 1996;20:241–6.
[13] Van Doorn LJ, Figueiredo C, Sanna R, Pena AS, Midolo P, Ng EK, et al. Expanding allelic diversity of H. pylori vacA. J Clin Microbiol 1998;36:2597–603.
[14] Kim SY, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, et al. Genotyping *cagA, vacA* subtype, *iceA1*, and *BabA* of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. J Korean Med Sci 2001;16:579.
[15] Kadi RH, Halawani EM, Abdelkader HS. Prevalence of *H. pylori* strains harboring cagA and iceA virulence genes in saudi patients with gastritis and peptic ulcer disease. Microbiol Discov 2014. doi: http://dx.doi.org/10.7243/2052-6180-2-2.
[16] Chisholm SA, Owen RJ, Teare EL, Saverymuttu S. PCR-based diagnosis of *Helicobacter pylori* infection and real-time determination of clarithromycin resistance directly from human gastric biopsy samples. J Clin Microbiol 2001;39:1217–20.
[17] Falsafi T, Favaedi R, Mahjoub F, Najafi M. Application of Stool-PCR test for diagnosis of *H. pylori* infection in children. World J Gastroenterol 2009;15:484–8.
[18] Goh KL. Prevalence of and risk factors for *H. pylori* infection in a multi-racial dyspeptic Malaysian population undergoing endoscopy. J Gastroenterol Hepatol 1997;12:29–35.
[19] Alazmi WM, Siddique I, Alateeqi N, Al-Nakib B. Prevalence of *H. pylori* infection among new outpatients with dyspepsia in Kuwait. BMC Gastroenterol 2010;10:14.
[20] Hasosah M, Satti M, Shehzad A, Alsahafi A, Sukkar G, Alzaben A, et al. Prevalence and risk factors of *H.pylori* infection in Saudi children: a three-year prospective controlled study. Helicobacter 2015;20:56–63.
[21] Zaki ME, Elewa A, Ali MA. Shehta A (2016): study of Virulence Genes *Cag A* and *Vac A* in *Helicobacter pylori* isolated from Mansoura University Hospital patients by multiplex PCR. Int J Curr Microbiol App Sci 2016;5(2):154–60.
[22] Ali AS, Borei MB. *H. pylori* and Egyptian infantile colic. J Egypt Soc Parasitol 2013;43:327–32.
[23] Abu-Zekry MA, Hashem M, Ali AA, Mohamed IS. Frequency of *H.pylori* infection among Egyptian children presenting with gastrointestinal manifestations. J Egypt Public Health Assoc 2013;88:74–8.
[24] Maleki P, LatifiNavid S, Zahri S. Allelic variation of Helicobacter pylori babA and cagA genes and their association with clinical consequences. Govaresh 2013;17:203–12.
[25] Eshraghian A. Epidemiology of *Helicobacter pylori* infection among the healthy population in Iran and countries of the Eastern Mediterranean Region: a systematic review of prevalence and risk factors. World J Gastroenterol 2014;20(46):17618–25.
[26] Peek RM, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002;2:28–37. doi: http://dx.doi.org/10.1038/nrc703.
[27] Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007;117:60–9. doi: http://dx.doi.org/10.1172/JCI30111.

[28] Ngoyi O, Nina E, Atipo Ibara BI, Moyen R, Apendi A, Clausina P, et al. Molecular detection of helicobacter pylori and its antimicrobial resistance in brazzaville, Congo. Helicobacter 2015;20:316–20.
[29] Rasheed F, Campbell BJ, Alfizah H, Varro A, Zahra R, Yamaoka Y, et al. Analysis of clinical isolates of *H. pylori* in Pakistan reveals high degrees of pathogenicity and high frequencies of antibiotic resistance. Helicobacter 2014;19:387–99.
[30] Kao C, Sheu B, Wu J, et al. Helicobacter pylori infection: an overview of bacterial virulence factors and pathogenesis. Bioomed. J. 2016;39:14–23.
[31] Yu J, Leung WK, Go MY, Chan MC, To KF, Ng EK, et al. Relationship between *Helicobacter pylori babA2*status with gastric epithelial cellturnover and premalignant gastric lesions. Gut 2002;51:480–4.
[32] Olfat FO, Zheng Q, Oleastro M, Voland P, Borén T, Karttunen R, et al. Correlation of the *Helicobacter pylori* adherence factor BabA with duodenal ulcer disease in four European countries. FEMS Immunol Med Microbiol 2005;44:151–6.
[33] Marie MA. Relationship between *H.pylori* virulence genes and clinical outcomes in Saudi patients. J Korean Med Sci 2012;27:190–3.
[34] Villalobos LB, Cavazza ME, Ortiz-Princz D. Detection of cagA gene and typing vacA gene of Helicobacter pylori in biopsies of patients with gastric symptoms in Cumana, Sucre State, Venezuela. Venezuela 2015;27(3): 430–440.
[35] Mattar R, Santos AF, Eisig JN, Rodrigues TN, Silva FM, Lupinacci RM, et al. No Correlation of babA2 with vacA and cagA Genotypes of *Helicobacter pylori* and Grading of Gastritis from Peptic Ulcer Disease Patients in Brazil. Helicobacter 2005;10:601–8.
[36] González-Vázquez A, Córdova-Espinoza MG, Escamilla-Gutiérrez A, Morales-Méndez I, Ochoa-Pérez SA, Armendáriz-Toledano F et al. Frequency of virulence genes in mixed infections with *Helicobacter pylori* strains from a Mexican population Revista de Gastroenterología de México 2016;81(1):11–20.
[37] Shiota S, Suzuki R, Yamaoka Y. The significance of virulence factors in *Helicobacter pylori*. J Digest Dis 2013;14(7):341–9.
[38] Amjad N, Osman HA, Razak NA, Kassian J, Din J, Abdullah NB. Clinical significance of Helicobacter pylori cagA and iceA genotype status". World J Gastroenterol 2010;16(35):4443–7.
[39] Boyanova N, Yordanov D, Gergova G, Markovska R, Mitov I. Association of iceA and babA genotypes in Helicobacter pylori strains with patient and strain characteristics". Antonie Van Leeuwenhoek 2010;98(3):343–50.
[40] Gatti LL, Módena JL, Payão SL, Smith Mde A, Fukuhara Y, Módena JL, et al. Prevalence of *Helicobacter pylori cagA*, *iceA* and *babA2* alleles in Brazilian patients with upper gastrointestinal diseases. Acta Trop 2006;100(3):232–40.
[41] Havaei A, Mohajeri P, Khashei R, Salehi R, Tavakoli H. Prevalence of *Helicobacter Pylori vacA* different genotypes in Isfahan. Adv Biomed Res 2014.
[42] Feliciano O, Gutierrez O, Valdés L, Fragoso T, Calderin AM, Valdes AE, et al. Prevalence of *Helicobacter pylori* vacA, cagA, and iceA Genotypes in Cuban patients with upper gastrointestinal diseases. BioMed Res Int 2015.
[43] Chattopadhyay S, Datta S, Chowdhury A, Chowdhury S, Mukhopadhyay AK, Rajendran K, et al. Virulence genes in *Helicobacter pylori* strains from West Bengal residents with overt *H.pylori*- associated disease and healthy volunteers. J Clin Microbiol 2002;40(7):2622–5. doi: http://dx.doi.org/10.1128/JCM. 40.7.2622-2625 [PubMed: 12089290].
[44] Van Doorn LJ, Figueiredo C, Mégraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of vacA allelic types of Helicobacter pylori. Gastroenterology 1999;116(4):823–30. doi: http://dx.doi.org/10.1016/S0016-5085(99)70065-X.
[45] Sandikci MU, Doran F, Koksal F, Sandikci S, Uluhan R, Varinli S, et al. *Helicobacter pylori* prevalence in a routine upper gastrointestinal endoscopy population. Br J Clin Pract 1993;47(4):187–9 [PubMed: 8260335].
[46] Massarrat S, Saberi-Firoozi M, Soleimani A, Himmelmann GW, Hitzges M, Keshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. Eur J Gastroenterol Hepatol 1995;7(5):427–33.
[47] Sugimoto M, Yamaoka Y. The association of *vacA* genotype and *Helicobacter pylori*–related disease in Latin American and African populations. Clin Microbiol Infect 2009;15:835–42.
[48] Cover TL, Tummuru MK, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. J Biol Chem 1994;269:10566e73.
[49] Falsafi T, Khani A, Mahjoub F, Asgarani E, Sotoudeh N. Analysis of *vacA/cagA* genotypes/status in *Helicobacter* pylori isolates from Iranian children and their association with clinical outcomeTurk. J Med Sci 2015;45:170–7. doi: http://dx.doi.org/10.3906/sag-1311-2.
[50] Chiurillo MA, Moran Y, Cãnas M, Valderrama E, Granda N, Sayegh M, et al. Genotyping of Helicobacter pylori virulenceassociated genes shows high diversity of strains infecting patients in western Venezuela. Int J Infect Dis 2013;17:e750e6.
[51] Sheu SM, Sheu BS, Yang HB, Li C, Chu TC, Wu JJ. Presence of iceA1 but not cagA, cagC, cagE, cagF, cagN, cagT, or orf13 genes of Helicobacter pylori is associated with more severe gastric inflammation in Taiwanese. J Formos Med Assoc 2002;101:18e23.
[52] Diab M, Saad El-Dine S, Aboul-Fadl L, Omran Z, Shemis M, El-Gannam M, et al. *H.pylori cag* pathogenicity island genes among dyspeptic patients with chronic gastritis. EJMM 2009;18:43–53.
[53] El-Fakhry AA, El-Daker MA, Badr RI, El-Nady GM, Mesbah MR, Youssef T, et al. Association of the *cagA* gene positive *H. pylori* and tissue levels of interleukin 17 and interleukin 8 in gastric ulcer patients. Egypt J Immunol 2012;19:51–62.
[54] Saribasak H, Salih BA, Yamaoka Y, Sander E. Analysis of Helicobacter pylori genotypes and correlation with clinical outcome in Turkey. J Clin Microbiol 2004;42:1648–51.
[55] Ferreira RM, Pinto-Ribeiro I, Wen X, Marcos-Pinto R, Dinis-Ribeiro M, Carneiro F, et al. *Helicobacter pylori* cagA promoter region sequences influence CagA expression and interleukin 8 secretion. J Infect Dis 2015. doi: http://dx.doi.org/10.1093/infdis/jiv467.
[56] Molaei M, Foroughi F, Mashayekhi R, Haghazali M, Zojaji H, Jafari F, et al. *CagA* status and *vacA* subtypes of *Helicobacter pylori* in relation to histopathologic findings in Iranian population. Indian J Pathol Microbiol 2010;53:24–7.
[57] Douraghi M, Saberi Kashani S, Shokrgozar MA, Oghalaie A, Esmaili M, Bababeik M, et al. Characterization of the vacuolating cytotoxin in *Helicobacter pylori* strains isolated from Iran. Cell J 2010;12:1–6.
[58] Momenah AM, Tayeb MT. Helicobacter pylori cagA and iceA genotypes status and risk of peptic ulcer in Saudi patients. Saudi Med J 2007;28:382–5.
[59] Ashour AA, Magalhaes PP, Mendes EN, Collares GB, de Gusmao VR, Queiroz DM, et al. Distribution of vacA genotypes in Helicobacter pylori strains isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma. FEMS Immunol Med Microbiol 2002;33:173–8.
[60] Ben Mansour K, Fendri C, Zribi M, Masmoudi A, Labbene M, Fillali A, et al. Prevalence of Helicobacter pylori vacA, cagA, iceA and oipA genotypes in Tunisian patients. Ann Clin Microbiol Antimicrob 2010;9:10.