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Detection of Cytotoxin-Associated and Vacuolating Cytotoxin Genotypes of Helicobacter pylori in Patients with Peptic Inflammatory/Ulcerative Disorders

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ABSTRACT

Background: *Helicobacter pylori* is a Gram negative, spiral, rod-shaped, and flagellated bacteria that colonizes the human gastric mucosa and can cause a strong inflammatory state and lesions. However, genomic and phenotypic features of different strains allow the expression of virulence factors which enable some strains, rather than others, to cause disease**. Aims:** The aim of the present study was to evaluate the role of the cytotoxicity genes, *CagA* and *VacA*, of *H. pylori* in patients with peptic inflammatory/ulcerative disorders and correlate between different genotypes and peptic lesions. **Patients and methods:** Upper gastrointestinal endoscopy and histopathological examination of gastric biopsy samples were done for 112 patients complaining of upper gastrointestinal symptoms and clinically suspected to have *H. Pylori* infection. *CagA* and *VacA* genotyping by PCR were done for 50 *H. pylori* +ve patients (diagnosed by histopathology). **Results:** *CagA* gene and *VacA* gene were detected in 42.0% and 70% of *H. pylori* +ve patients respectively. There was a significant positive correlation between *CagA* and duodenal erosion and ulceration visualized by endoscopy. There was also a positive correlation between *CagA* and gastric erosion and ulceration visualized by endoscopy, but it didn`t reach a significant level. There was also a significant positive correlation between the *VacA m1s1* subtype and duodenal erosion and ulceration detected by endoscopy. *VacA m2s2* was correlated to presence of both gastric erosions and ulcerations and presence of metaplasia and atrophy. There was only one *H. pylori* +ve patient with gastric cancer. This patient was positive for both *CagA* and *VacA m2s2*. **Conclusions:** The *CagA* gene is associated with severe forms of gastric pathology (peptic ulcer disease "PUD" and precancerous gastric lesions) and the *VacA m2s2* subtype is associated with variable forms of gastric pathology rather than other *VacA* gene subtypes. **Recommendations:** Genotyping for *VacA* and *CagA* of *H. pylori* infected patients is helpful to determine the patients at more risk. Further studies are needed to evaluate the virulence factors in *H. pylori* with emphasis on role of *CagA* and *VacA m2s2* both in vivo and in vitro.

Keywords: Cytotoxin-Associated, Vacuolating Cytotoxin, Genotypes, Helicobacter pylori, Peptic Inflammatory, Ulcerative Disorders.

INTRODUCTION

Helicobacter pylori is a Gram negative, spiral, rod-shaped, and flagellated bacteria that colonizes the human gastric mucosa and can cause a strong inflammatory state and lesions. However, genomic and phenotypic features of different strains allow the expression of virulence factors which enable some strains, rather than others, to cause disease[1]**.** The *CagA* is toxic to the cell by different mechanisms including induction of cellular hyper proliferation, apoptosis and failure of gastric epithelial cell ability to maintain its normal cytoskeletal structure, an important prerequisite for neoplastic transformation[2]**.** *VacA* is the second most extensively studied *H. pylori* virulence factor. In addition to inducing vacuolation, *VacA* can induce multiple cellular activities, including membrane-channel formation, cytochrome c release from mitochondria leading to apoptosis, and binding to cell-membrane receptors followed by initiation of a proinflammatory response[3]**.** Most studies about the association between genotypes of *H. pylori* and chronic gastritis, peptic ulcer (PU) disease have been conducted in the Western populations, and very few studies had examined these associations in the Middle East. However, there appears to be a geographic variation in the association between *H. pylori* genotypes and gastric inflammatory response[4]**.** In this work we are willing to study the frequency of *H. pylori CagA* and *VacA* genotypes and their correlation to gastroduodenal lesions. So that this cross sectional study aimed to evaluate the role of the cytotoxicity genes, *CagA* and *VacA*, of *Helicobacter pylori* in patients with peptic inflammatory/ulcerative disorders and correlate between different genotypes and peptic lesions.

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PATIENTS AND METHODS

During the period between September 2015 and December 2017, this study was conducted on 112 patients complaining of upper gastrointestinal (GI) symptoms and clinically suspected to have *H. Pylori* infection. Patients were subjected to upper GI endoscopy (Olympus X Q40) at Gastrointestinal Endoscopy Unit, October Clinic Hospital, Giza, Egypt and all patients provided a written informed consent.

The study was approved by the Ethics Board of Al-Azhar University.

Patients with a recent history of antibiotics, bismuth salts or proton pump inhibitor intake in the preceding four weeks were excluded. Rapid urease test (Campylobacter-like organism (CLO) test, Halyard health Inc., USA) was done for only 51 patients` gastric antral biopsies. It was done at Gastrointestinal Endoscopy Unit, October Clinic Hospital, Giza, Egypt. Histopathological examination, using Giemsa and hematoxylin and eosin (H&E) stains, was done for, formalin-fixed, paraffin-embedded, gastric biopsy samples. Samples for histopathology were taken from gastric antrum of all patients. For 15 patients, other gastric biopsy specimens for histopathology were obtained from gastric gross lesions to confirm their diagnosis. Histopathology was done at Pathology Department, Faculty of Medicine, Al-Azhar University. The diagnosis of duodenal lesions was dependent on endoscopy, while, the diagnosis of gastric lesions was dependent on both endoscopy and histopathology.

Using specific primers (Table 1), detection of *CagA* and *VacA* genotypes by PCR was done for 50 gastric antrum biopsy samples taken from 50 patients diagnosed by histopathology as positive for *H. pylori* colonization. Samples were stored at -80^{o}C.

DNA extraction by Geneaid tissue genomic DNA Mini kit (Geneaid Biomed Ltd., Taiwan), genes amplification by thermal cycler (Biometra UNO-Thermoblock, Analytik Jena, Botron, Germany) and detection by agarose gel electrophoresis were done at Molecular Biology Unit, Microbiology and Immunology Department, Faculty of Medicine, Al-Azhar University.

Genomic DNA extraction was done following manufacturer guidelines. PCR assay was performed in a volume of 20 ul with approximately 2 ug of extracted DNA, 0.6 µl of (each) primer, 4 µl 5x FIREPol ready to load master mix (Solis BioDyne). For PCR amplification conditions, the following cycling parameters were used; denaturing at 94°C for 4 min followed by 30 cycles of, denaturation at 94°C for 1 min, an annealing at 54°C for *CagA* and 52°C for *VacA*, and elongation at 72°C for 1 min, followed by a final extension of 72°C for 10 min. Each PCR product was separated on 2% agarose gel with ethidium bromide, and a 50 bp ladder was used as DNA molecular weight standard. In each PCR assay, a negative control (lacking DNA) was included.

Table 1: Primers for *CagA* and *VacA* genes for PCR assays[5]

Target genes		Primers sequence(5\-3\)	Amplicon bp
CagA		5'AAT ACA CCA ACG CCT CCA-3' 5'TTG TTG CCG CTT TTG CTC TC-3'	**400**
VacA	***VacA (s1/s2)***	5'ATG GAA ATA CAA CAA ACA CAC-3' 5'CTG CTT GAA TGC GCC AAA C-3'	**259/286**
	VacA (m1/m2)	5'CAA TCT GTC CAA TCA AGC GAG-3' 5'GCG TCT AAA TAA TTC CAA GG-3'	**570/642**

Statistical analysis

Data were collected, revised and entered using the statistical package SPSS version 22. The collected data were tabulated and analyzed with the suitable statistical methods using mean value ± standard deviation, t-test, z-test and chi square test. P value of less than 0.05 was considered statistically significant.

RESULTS

This study was carried out on 112 patients were complaining of upper gastrointestinal symptoms and clinically suspected to have *H. pylori* infection, in the period between September 2015 and December 2017. They were 74 males and38 females, and their ages ranged between 17 and 75 year **(Table 2).**

Table (2): Demographic features of the patients included within the study.

Demographic features		**Patients` study**
		No. = 112
Age	Range	17 – 75
	Mean±SD	39.73 ± 12.58
Sex	Male	74 (66.1%)
	Female	38 (33.9%)
Residence	Rural	66 (58.9%)
	Urban	46 (41.1%)

Table (3): Endoscopic and histopathological findings in all studied patients.

Pathology		**Gastroscopy**		**histopathology**		**P-value**
		No.= 112	**100%**	**No.= 112**	**100%**	
Normal gastric mucosa		60	53.6%	23	20.5%	<0.001*
Acute gastritis		0	0.0%	0	0.0%	-
Chronic gastritis		52	46.4%	74	66.1%	<0.001*
Gastric Mucosal lesions	**Mild gastritis**	15	13.4%	45	40.2%	<0.001*
	Moderate gastritis	14	12.5%	23	20.5%	0.105
	Severe gastritis	8	7.1%	6	5.4%	0.582
	Gastric erosion	7	6.3%	7	6.3%	1.0
	Gastric ulcer	6	5.4%	6	5.4%	1.0
	Gastric cancer	2	1.8%	2	1.8%	1.0
Intestinal metaplasia	**Mild**	-	-	1	1.9%	-
	Moderate	-	-	3	2.7%	-
	Severe	-	-	0	0.0%	-
Glandular atrophy	**Mild**	-	-	18	16.1%	
	Moderate	-	-	7	6.2%	-
	Severe	-	-	0	0.0%	
Lymphocyte follicles formation		-	-	5	4.5%	-

*P value ≤0.05: significant.

Table (3) shows that there were highly significant variations between gastroscopy and histopathology in relation to both normal gastric mucosa and gastritis (P-values <0.001). However, there were no variations between them in relation to either gastric erosion, ulcer or cancer.

In the present study *Helicobacter pylori* was detected by histopathology in 50 (44.6%) of the total patients studied (Figure 1).

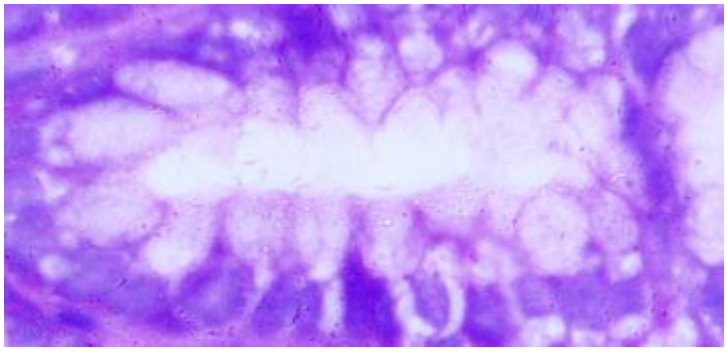

Figure (1): Antral mucosa with *H. pylori* colonization (Giemsa, ×1000)

Rapid urease test (CLO) test was done for only 51 patients` gastric antral biopsies. It was found that 18 out of 25 *H. pylori* +ve samples (72%) were positive by rapid urease (CLO) test while 7 (28%) of *H. pylori* +ve samples were found negative. This difference was highly significant (P-value <0.001).

Table No. (4): Comparison between *H. pylori* +ve and *H. pylori* –ve patients regarding patients` age, sex and residence.

Demographic features		***H. pylori* +ve No. = 50**	***H. pylori* -ve No. = 62**	**P-value**
Age	**Range**	19 – 65	17 – 75	
	Mean±SD	39.38 ± 10.94	40.02 ± 13.84	0.791
Sex	**Male(74)**	35 (47.3%)	39 (52.7%)	0.430
	Female(38)	15 (39.5%)	23 (60.5%)	
Residence	**Rural (66)**	32 (48.5%)	34 (51.5%)	0.327
	Urban (46)	18 (39.1%)	28 (60.9%)	

***P value ≤0.05: significant.**

It was found that there was no statistically significant difference as regard age, sex and residence in both *H. pylori* +ve and *H. pylori* –ve patients (Table 4)

Table (5): Endoscopic and histopathological findings in relation to *Helicobacter pylori* detection by histopathology.

Findings			***H. pylori* +ve No.=50**	***H. pylori* -ve No.=62**	**P-value**
Endoscopic	Normal gastric mucosa (60)		28 (46.7%)	32 (53.3%)	0.645
	Gastritis (37)	Mild (15)	5 (33.3%)	10 (66.7%)	0.548
		Moderate(14)	5 (35.7%)	9 (64.3%)	
		Severe (8)	4 (50%)	4 (50%)	
	Gastric erosion (7)		3 (42.9%)	4 (57.1%)	0.920
	Gastric ulcer (6)		4 (66.7%)	2 (33.3%)	0.265
	Gastric cancer (2)		1 (50%)	1 (50%)	0.878
	Normal duodenal mucosa (84)		35 (41.7%)	49 (58.3%)	0.351
	Duodenitis (8)	Mild (6)	4 (66.7%)	2 (33.3%)	0.271
		Moderate (1)	0 (0%)	1 (100%)	
		Severe (1)	1 (100%)	0 (.0%)	
	Duodenal erosion (12)		6 (50%)	6 (50%)	0.907
	Duodenal ulcer (8)		4 (50%)	4 (50%)	0.752
Histopathological	Chronic gastritis(74)		34 (68.0%)	40 (64.5%)	0.286
	ensity of inflammation	Mild(45)	19 (42.2%)	26 (57.8%)	0.399
		Moderate(23)	11 (42.2%)	12 (52.2%)	
		Severe(6)	4 (66.7%)	2 (33.3%)	
	Gastric erosion (7)		3 (6%)	4 (6.5%)	0.920
	Gastric ulcer (6)		4 (8%)	2 (3.2%)	0.265
	Gastric cancer (2)		1 (2%)	1 (1.6%)	0.878
	Intestinal metaplasia(4)	Mild (1)	1 (2.0%)	0 (0.0%)	0.398
		Moderate(3)	2 (4.0%)	1 (1.6%)	
		Severe(0)	0 (0.0%)	0 (0.0%)	
	Glandular atrophy (25)	Mild(18)	12 (24.0%)	6 (9.7%)	0.088
		Moderate(7)	4 (8.0%)	3 (4.8%)	
		Severe(0)	0 (0.0%)	0 (0.0%)	
	Lymphocyte follicles formation(5)		5 (10.0%)	0 (0.0%)	0.011*

*P-value ≤ 0.05: Significant

Table (5) demonstrated that gastroscopic and duedenoscopic findings were not statistically related to presence of *H. pylori* in histopathological samples. Whereas presence of lymphocyte follicles by histopathology were significantly associated with *H. pylori* detection (P-value < 0.05).

Table (6): Toxin genes by PCR in *Helicobacter pylori* positive patients

Positivity of toxin genes				Negative for both toxin genes	Total samples studied for toxin genes
***CagA* only**	***VacA* only**	**Both *VacA* and *CagA***	**Total**		
0 (0%)	**14 (28%)**	**21 (42%)**	**35 (70%)**	**15 (30%)**	**50 (100%)**

Table (6) showed that *VacA* gene alone was detected in 14 samples (28%), while both *VacA* and *CagA* genes were detected simultaneously in 21 samples (42%) of the total 50 *H. pylori* +ve patients.

Table (7): Description of *Helicobacter pylori* genotypes for the toxin genes detected by PCR

Genotypes	*CagA*	*VacA*				
	Positive	*m1s1*	*m1s2*	*m2s1*	*m2s2*	Total
No.= 50	21	6	0	11	18	35
%	42 %	12%	0%	22%	36%	70%

Table(7) shows that *CagA* gene was detected in 21 patients (42.0%) and *VacA* gene was detected in 35 patients (70%) with the *m1* (6 (17.1%)), *m2* (29 (82.9%)), *s1* (17 (48.6%)) , *s2* (18 (51.4%)). As regard *VacA* subtypes, *m1s1* was detected in 6 patients (12%), *m2s1* in 11 patients (22%) and *m2s2* in 18 patients (36%). However, the subtype *m1s2* was not detected in any sample studied (Figure 2 and 3).

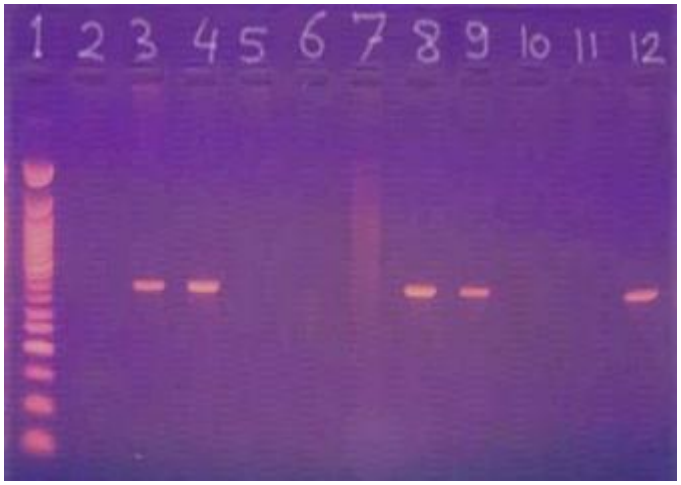

Figure (2): **Agarose gel electrophoresis for the amplicons of the *CagA* gene:** Lane 1 contains the DNA ladder (50 bp) and lane 2 contains negative control. While, lanes 3, 4, 8, 9 and 12 contain the 400 bp *CagA* gene. However, lanes 5, 6, 7, 10 and 11 contain negative samples for the *CagA* gene

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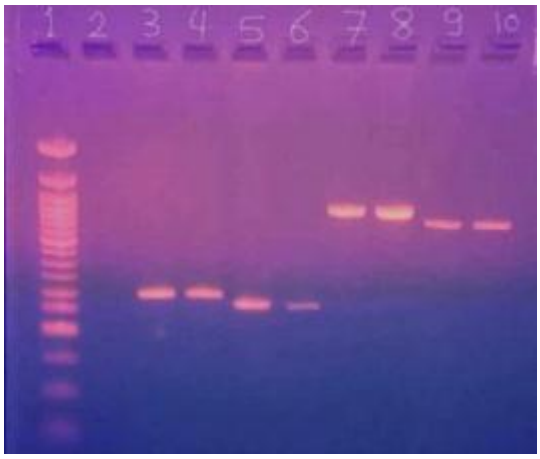

Figure (3): **Agarose gel electrophoresis for the amplicons of different *VacA* subtypes:** Lane 1 contains the DNA ladder (50 bp) and lane 2 contains negative control. While, lanes 9 and 10 contain the 570 bp *VacA m1* gene, lanes 7 and 8 contain the 642 bp *VacA m*2 gene, lanes 5and 6 contain the 259 bp *VacA s1* gene and lanes 3 and 4 contain the 286 bp *VacA S2* gene.

Table (8): Association between different *VacA* gene subtypes and *CagA* gene in *H. Pylori* +ve patients.

***VacA* subtypes**	***CagA +ve* (No.= 21)**	***CagA -ve* (No.= 29)**	**P-value**
***VacA m1s1* (No.= 6)**	6 (100.0%)	0 (0.0%)	0.028*
***VacA m2s1* (No.= 11)**	7 (63.6%)	4(36.4%)	0.766
***VacA m2s2* (No.= 17)**	7 (41. 2%)	10 (58.8%)	-0.027*

*** P value ≤0.05= significant.**

Table (8) revealed that *VacA m1s1* subtype was significantly positively associated with *CagA*. On the other hand, *VacA m2s2* subtype showed a significant negative association to *CagA* gene.

Table (9): Correlation between cytotoxin genes (*CagA / VacA)* and endoscopic / histopathological findings in *H. Pylori* +ve patients

Abnormal findings in 50 *H. pylori* +ve patients		***CagA***	***VacA***	**P- value**	
		No.= 21 (42%)	**No.= 35 (70%)**	**[1]P**	**[2]P1**
Gastric lesions	**Gastritis (34)**	14 (41. 2%)	25 (73.5%)	0.863	0.427
	Gastric erosion/ulceration (7)	5 (71.4%)	6 (85.7%)	0.089	0.328
	Gastric cancer (1)	1 (100.0%)	1 (100.0%)	0.235	0.508
Duodenal lesions	**Duodenitis (5)**	1 (20.0%)	3 (60.0%)	0.293	0.607
	Duodenal erosion/ulceration (10)	7 (70.0%)	8 (80.0%)	0.045*	0.440
Other histopathological findings	**Lymphocyte follicle formation (5)**	5 (100.0%)	5 (100.0%)	0.006*	0.123
	Intestinal metaplasia (3)	3 (100.0%)	3 (100.0%)	0.036*	0.242
	Glandular atrophy (16)	10 (62.5%)	14 (87.5%)	0.044*	0.064

1-P values for *CagA* relations. 2- P values for *VacA* relations. * P value ≤0.05= significant.

Table (9) revealed that there was a significant positive correlation between *CagA* detection and duodenal erosive/ulcerative disorders visualized by endoscopy (P-value < 0.05). There was a positive correlation between *CagA* detection and gastric erosive/ulcerative disorders visualized by endoscopy, however it didn`t reach a significant level. There were significant positive correlations between *CagA* detection and intestinal metaplasia, glandular atrophy and lymphocyte follicles formation detected by histopathology (P-value <0.05).

Table (10): Correlation between *VacA* gene subtypes and abnormal gastroduodenal /histopathological findings.

Abnormal findings in 50 *H. pylori* +ve patients		*VacA m1s1* No.= 6 (12%)	*VacA m2s1* No.= 11 (22%)	*VacA m2s2* No.= 18 (36%)	P- value [3]P2	[4]P3	[5]P4
Gastric lesions	Gastritis (34)	6 (17.6%)	9 (26.5%)	10 (29.4%)	0.073	0.266	0.157
	Gastric erosion/ulceration (7)	0 (0.0%)	1 (14.3%)	5 (71.4%)	0.292	0.595	0.024*
	Gastric cancer (1)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0.709	0.592	0.159
Duodenal lesions	Duodenitis (5)	0 (0.0%)	0 (0.0%)	3 (60.0%)	0.384	0.211	0.196
	Duodenal erosion/ulceration (10)	3 (30.0%)	2 (20.0%)	2 (20.0%)	0.050*	0.864	0.296
Other histopathological findings	Lymphocyte follicle formation (5)	1 (20.0%)	1 (20.0%)	2 (40.0%)	0.562	0.909	0.765
	Intestinal metaplasia (3)	0 (0.0%)	0 (0.0%)	3 (100.0%)	0.509	0.343	0.013*
	Glandular atrophy (16)	2 (12.5%)	1 (6.3%)	10 (62.5%)	0.941	0.065	0.004*

3- P values for *VacA m1s1* relations. 4-P valuesfor*VacAm2s1* relations.
5- P values for *VacA m2s2* relations * P value ≤0.05: significant.

Table (10) shows that there was a significant positive correlation between the *VacA m1s1* subtype and duodenal erosion and ulceration detected by endoscopy (P-value < 0.05). There was a positive correlation between the *VacA m1s1* subtype and gastritis detected by histopathology, however, it didn`t reach a significant level .There was a significant positive correlation between *VacA m2s2* detection and presence of gastric erosions and ulcerations visualized by endoscopy (P-value < 0.05). There were significant positive correlations between *VacA m2s2* and presence of intestinal metaplasia and glandular atrophy detected by histopathology (P-values < 0.05).

Table (11): Comparison between *Vac+/ Cag+* and *Vac+/ Cag- H. pylori* genotypes as regard abnormal endoscopic and histopathological findings

Abnormal findings in 35 *H. pylori* +ve patients		*Vac+/ Cag+* No.= 21	%	*Vac+/ Cag-* No.= 14	%	P-value
Gastric lesions	Gastritis (26)	14	53.8%	12	46.2%	0.207
	Gastric Erosion/ ulceration (6)	5	83.3%	1	16.7%	0.200
	Gastric cancer (1)	1	100.0%	0	0.0%	0.407
Duodenal lesions	Duodenitis (3)	1	66.7%	2	33.3%	0.324
	Duodenal Erosion/ ulceration (8)	7	87.5%	1	12.5%	0.071
Other histopathological abnormalities	Lymphocyte follicles formation (5)	5	100.0%	0	0.0%	0.049*
	Intestinal metaplasia (3)	3	100.0%	0	0.0%	0.139
	Glandular atrophy (16)	10	71.4%	4	28.6%	0.260

* P value ≤0.05= significant.

Table(11) shows that formation of lymphocyte follicles that detected by histopathology was significantly more frequent in patients positive for both *CagA* and *VacA* genes than patients positive for *VacA* only (P-value < 0.05). Other abnormal endoscopic and histopathological findings, were detected more frequently in patients positive for both genes, but they didn`t reach significant levels.

DISCUSSION

In the present study, *H. pylori* was detected by histopathological examination of biopsies taken from the gastric antrum. The advantages of histopathology include its ability to document *H. pylori* infection with 100% specificity and provides more information about the degree of inflammation and associated pathology, such as, atrophic gastritis, intestinal metaplasia, gastric cancer, or lymphoma. Hematoxylin and eosin staining is usually adequate and Giemsa stain seems to have advantage over other stains because of its simplicity and consistency[6].

Conventionally, the site of gastric biopsy used by most endoscopists is the antrum with excellent sensitivity and specificity (over 90%). Antral biopsy has been reported to be more sensitive in the detection of *H. pylori* when compared to that of the corpus[7].

In the present study, we found significant correlations between endoscopy and histopathology in diagnosis of gastric erosion, ulcer and cancer. Near to our results, ***Kazi et al.***[8] who found a good correlation between endoscopy and histopathology in cases of gastric ulcer. While, ***Sharma et al.***[9] found a good correlation in cases of gastric cancer. On the other hand, we found no correlation between endoscopy and histopathology in either cases with normal gastric mucosa or gastritis. ***Kazi et al.***[8] found a poor correlation to histopathology when the endoscopy revealed no abnormality and found that gastritis was one of the most commonly missed lesions on endoscopy. So that, histopathology is essential in order to document a normal gastric mucosa. Also, ***Sharma et al.***[9]found a poor correlation between endoscopic and histopathological evidence of gastritis**.**

Although *H. pylori* infection occurs worldwide, there are significant differences in its prevalence both within and between countries[10]**.**

In the present study, *H. pylori* was detected by histopathology in 44.6% of the total patients studied. Whereas, ***El-Shenawy et al.***[11]**,** ***Boukhris et al.***[12] and ***Siddique et al.***[4] found that *H. pylori* was detected in 53.1%, 63.7% and 81.6% respectively. The relatively low frequency of *H. pylori* in this study may be related to number of cases enrolled and may be due to the strict exclusion criteria by which an acid reducing therapy had to be discontinued for at least 4 weeks, which may be a relatively long period.

In the current study, 28% of *H pylori* +ve specimens (detected by histopathology) were found negative by CLO test. This finding was near to that reported by ***Tahir et al.***[13] who found false negative CLO test in (43.6%) and of *H pylori* +ve samples. A possible explanation is that positive CLO test requires approximately 105 CFU of *H. pylori* in the biopsy sample. The organisms tend to localize on or near the surface of the specimen, such that most of the tissue is ‘‘extra” and does not contribute to the reaction. Therefore, it is better to use an opened forceps to scrape gastric mucosa to ensure that a high concentration of bacteria-rich material is obtained. Other common causes of false negative test are the presence of intestinal metaplasia which is often devoid of *H. pylori*, or obtaining a small biopsy[14]**.**

Regarding endoscopic findings in the 50 *H. pylori* +ve patients in this study, the most common abnormal findings were gastritis (14/50, 28%), peptic erosion (9/ 50, 18%) and peptic ulcer (8/ 50, 16%). This finding was in agreement with ***Kong et al.***[15] who reported that gastritis is the first and the most prevalent lesion observed in *H. pylori* infection.

Regarding histopathology, we found that chronic inflammation was more frequent and severe in *H. pylori* +ve patients, however, with no statistical significance. Whereas, ***Boukhris et al.***[12] reported a significant rise in frequency of chronic inflammation within *H. pylori* +ve patients.

Chronic gastritis caused by *H. Pylori* infection can progress to more severe lesions such as intestinal metaplasia and glandular atrophy**.** In this study, intestinal metaplasia and glandular atrophy were found more frequently in *H. pylori* +ve patients, however, with no statistical significance. On the other hand, ***Boukhris et al.***[12] reported that *H. pylori* was significantly associated with glandular atrophy but negatively associated with intestinal metaplasia.

The normal gastric mucosa contains no or very few lymphocytes in the lamina propria. Lymphoid follicles and aggregates are characteristic of H pylori associated gastritis[16]**.**

In the present study, we found that presence of lymphocyte follicles was significantly associated with *H. pylori* infection. Along with this result, ***Kiewicz and Kobierska***[17] found a close relationship between lymphoid follicles formation and *H. pylori* infection, particularly in younger patients. Lymphoid follicles within gastric mucosa of *H. pylori* infected patients are considered a mucous associated lymphoid tissue (MALT) which in turn a possible precursor lesions for MALT lymphoma[17]**.**

In the current study, the *CagA* gene was detected in 42.0% of *H. pylori* +ve patients. This finding was similar to ***Meine et al.***[1] (42.5%). Lower result (26.6%) was obtained by ***El-Shenawy et al.***[11]**.** Whereas**,** ***Boukhris et al.***[12] and ***Siddique et al.***[4] detected *CagA* gene in 59.6% and 52.5%, of *H. pylori* positive patients respectively. However, higher results were obtained by ***Zhang et al.***[18] (98%) and ***Kadi et al.***[19] (81.7%) of *H. pylori* positive patients.

The other cytotoxin gene, *VacA*, encodes a vacuolating toxin that is excreted by *H. pylori*. In this study, *VacA* gene was detected in 70% of *H. pylori* +ve patients. Lower result (61.6%) was obtained by ***El-Shenawy et al***[11]**.** However, higher results were obtained by ***Boukhris et al.***[12] and ***Siddique et al.***[4] who detected *VacA* gene in 84.3% and 93.9 % of *H. pylori* +ve patients respectively.

As regard *VacA* subtypes, we found that the commonest genotype was *m2s2* (36%). While, *m2s1* and *m1s1* were detected in 22% and 12% of *H. pylori* +ve patients respectively. Whereas, the subtype *m1s2* was not detected in any patient. Very close to these results, ***Boukhris et al.***[12] and ***El-shenawy et al.***[11] who found that the most common gene combinations of the *VacA* was the *m2s2* genotype (24.7% and 51.4% respectively). While, *m1s1* was detected in 16.9% and 27% and *m2s1* in 6.9% and 18.9% of *H. pylori* infected patients respectively. Whereas, they detected *m1s2* genotype in only one *H. pylori* infected patient (0.8% and 2.7% respectively).

The variations in frequencies of *VacA* genotypes and *CagA* gene in *H. pylori* isolates from different parts of the world may be related to the genetic heterogeneity, in addition to using different gene primers[20]**.** Striking genetic variability at the level of and within single genes (microvariability) has been also noted in *VacA* and *CagA*[21]**.**

In the current study, we found a significant positive correlation between detection of *CagA* gene and duodenal erosion and ulceration. There were also positive correlation between presence of *CagA* and gastric erosion and ulceration, but it didn`t reach a significant level. These results were near to that obtained by ***Siddique et al.***[4] and ***Kadi et al.***[19] who found a strong correlation between detection of *CagA* gene and both gastric and duodenal ulcer.

Blazer and Atherton[22] documented that persons with *CagA* are more likely to develop intestinal metaplasia, glandular atrophy and gastric cancer. In this study, we found correlations between *CagA* gene and presence of the precancerous lesions, glandular atrophy and intestinal metaplasia, detected by histopathology. These findings were also obtained by ***Meine et al.***[1]**.** On the other hand, ***Boukhris et al.***[12] didn`t find these correlations.

In the present study we found only one *H. pylori* +ve patient with gastric cancer. This patient was positive for both *CagA* and *VacA m2s2*. ***Meine et al.***[1]**,** ***Boukhris et al.***[15] and ***Zhang et al.***[23] found an association between *CagA*- positive *H. pylori* infection and presence of gastric cancer. However, the current study failed to achieve a correlation between *CagA* gene and gastric cancer. This may be due to a relatively small number of cases enrolled in this study.

As regard *VacA* allele combination, ***Ferreira et al.***[23] reported that *VacA m1s1* was found to be more virulent than *m2s2* with association to PU, premalignant lesions and gastric cancer, as it produces high levels of vacuolating cytotoxin in vitro. In the present study, we found that *VacA m1s1* was correlated only to duodenal erosion and ulceration detected by endoscopy. This could be explained by the relatively lower frequency of *VacA m1s1* genotype in our study (12% vs. 36% for *m2s2*) with subsequent failure to obtain significant correlations to various endoscopic or pathological findings. On the other hand, we found that *VacA M2S2* was correlated to presence of both gastric erosions and ulcerations and presence of intestinal metaplasia and glandular atrophy. Whereas, in vitro experiments demonstrated that strains with genotype *m2s2* produces an inactive or no detectable cytotoxin[24]**.** However, this may be not precisely related to *H. pylori* strains isolated from our region. Also in the population of this study, triggering factors (environmental, genetic, etc.) for conversion of the inactive *m2s2* cytotoxin to an active form, may be considered as another possibility.

On the other hand, the severer lesions detected in correlation to *VacA m1s1* in other studies may be a reflection of the *CagA* gene effect which may coexist within the same strain of the *VacA m1s1* subtype. This possibility becomes higher in regions with high prevalence of the *CagA* gene. However, we did not find such coexistence between *CagA* and *m2s2* in our study.

In the present study, we found that formation of lymphocyte follicles detected by histopathology was significantly more frequent in patients positive for both *CagA* and *VacA* genes than patients positive for *VacA* only. Whereas, intestinal metaplasia and gastric cancer were detected only in patients infected with *Vac+/ Cag+ H. pylori* genotype. Apart from duodenitis, other abnormal endoscopic and

histopathological findings, were detected more frequently in patients infected with *Vac+/ Cag+ H. pylori* genotype, however, they didn`t reach a significant level. ***Olfat et al.***[25] reported that *Vac+/ Cag+* genotype is considered to be more virulent. Along with this, ***El-Shenawy et al.***[11] found more severe lesions in patients infected with *Vac+/ Cag+ H. pylori* genotype.

CONCLUSIONS AND RECOMMENDATIONS

The frequency of *H. pylori* infection and the associated cytotoxin genes varies greatly from one region to another. *CagA* gene is associated with severe forms of gastric pathology (PUD and precancerous gastric lesions, Intestinal metaplasia and glandular atrophy). On the other hand, the *VacA m2s2* subtype is associated with variable forms of gastric pathology rather than other *VacA* gene subtypes. However, this may be either due to change of the genotype behavior (to become more virulent) or due to relatively lower frequency of the commonly known to be the most virulent *VacA* genotype, the *m1s1*.

We recommend:

Requesting histopathological examination for patients undergoing upper GI endoscopy to avoid missed peptic pathology.

Regarding CLO test, scrapping the gastric mucosa and proper immersion of the gastric biopsy into the agar is important to avoid false negative results. Also, insure adequate contact time for the CLO test to avoid either very early negative or very late positive results.

Genotyping for *VacA* and *CagA* of *H. pylori* infected patients is helpful to determine the patients at more risk.

Further studies are needed to evaluate the virulence factors of *H. pylori* with emphasis on the role of *CagA* and *VacA m2s2* both in vivo and in vitro.

REFERENCES

1. **Meine GC, Rota C, Dietz J et al.(2011):** Relationship between cagA-positive Helicobacter pylori infection and risk of gastric cancer: a case control study in Porto Alegre. Brazil Arq Gastroenterol., 48(1):41-45.
2. **Minohara Y, Boyd D, Hawkins HK et al.(2007):** The effect of the cag pathogenicity island on binding of Helicobacter pylori to gastric epithelial cells and the subsequent induction of apoptosis. Helicobacter., 12:583-590.
3. **Kusters JG, van Vliet AH and Kuipers EJ (2006):** Pathogenesis of H. pylori infection. Clin Microbiol Rev., 19:449–490.
4. **Siddique I, Al-Qabandi A, Al-Ali J et al.(2014):** Association between Helicobacter pylori genotypes and severity of chronic gastritis, peptic ulcer disease and gastric mucosal interleukin-8 levels: Evidence from a study in the Middle East. Gut Pathogens, 6:41.
5. **Falsafi T, Favaedi R, Mahjoub F et al.(2009):** Application of Stool-PCR test for diagnosis of H. pylori infection in children. World J Gastroenterol., 15:484-488.
6. **Lee JY and Kim N (2015):** Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med., 3(1):10.
7. **Dursun M, Yilmaz S, Yukselen V et al.(2004):** Evaluation of optimal gastric mucosal biopsy site and number for identification of Helicobacter pylori, gastric atrophy and intestinal metaplasia. Hepatogastroenterology, 51:1732–1735.
8. **Kazi J, Alam M, Kazi A et al.(1990):** Correlation of endoscopic and histological diagnosis in upper gastrointestinal lesions. J pak Med Assoc., 40(12):281-283
9. **Sharma S, Makaju R, Dhakal R et al.(2015):**Correlation between endoscopic and histopathological findings in gastric lesions. Kathmandu Univ Med J., 13(51): 216-219
10. **Goodman KJ and Correa P (1995):** The transmission of Helicobacter pylori. A critical review of the evidence. International Journal of Epidemiology, 24(5):875–887.
11. **El-Shenawy A, Diab M, Shemis M et al.(2017):** Detection of Helicobacter pylori vacA, cagA and iceA1 virulence genes associated with gastric diseases in Egyptian patients. The Egyptian Journal of Medical Human Genetics, 18:365–371.
12. **Boukhris SA, Amarti A, El Rhazi K et al.(2013):** Helicobacter pylori genotypes associated with gastric histo-pathological damages in a Moroccan population. PLoS ONE, 8(12):e82646.
13. **Tahir F, Arshad S, Evans RC et al.(2012):**Higher than expected false negative CLO test in patients not taking PPI associated with regular alcohol intake and absence of endoscopic gastritis, Gut, doi:10.1136/gutjnl-2012-302514b.219
14. **Graham DY and Miftahussurur M (2018)** Helicobacter pylori urease for diagnosis of Helicobacter pylori infection: A mini review. J Adv Res., https://doi.org/10.1016/j.jare.2018.01.006
15. **Kong Y, Yi H, Dai J et al.(2014):** Histological changes of gastric mucosa after Helicobacter pylori eradication: a systematic review and meta-analysis. World J Gastroenterol., 20(19): 5903-5911.
16. **Dixon MF, Genta RM, Yardley JH et al.(1996):** Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis. Am J Surg Pathol., 20:1161–1181.

17. **Kiewicz K and Kobierska G (2000)**: Lymphoid aggregates in gastric biopsies: relationship to other mucosal lesions. Archivum Immunologiae et Therapiae Experimentalis, 48:201–204.
18. **Zhang Z, Zheng Q, Chen X et al.(2008):** The Helicobacter pylori duodenal ulcer promoting gene, dupA, in China. BMC Gastroenterol., 32(8):49–54.
19. **Kadi RH, Halawani EM and Abdelkader HS (2014):** Prevalence of H. pylori strains harbouring cagA and iceA virulence genes in saudi patients with gastritis and peptic ulcer disease. Microbiology Discovery, 23(6):85–87.
20. **Covacci A, Telford JL, Del Giudice G et al.(1999)**: Helicobacter pylori virulence and genetic geography. Science, 284:1328–1333.
21. **Göttke M, Fallone C, Barkun A et al.(2000):** Genetic variability determinants of Helicobacter pylori: influence of clinical background and geographic origin of isolates. The Journal of Infectious Diseases, 181(5):1674–1681
22. **Blazer JM and Atherton CJ (2004)**: Helicobacter pylori: Biology and disease. J Clin Invest., 113: 321-333.
23. **Ferreira RM, Machado JC and Figueiredo C (2014):** Clinical relevance of Helicobacter pylori vacA and cagA genotypes in gastric carcinoma. Best Practice & Research Clinical Gastroenterology, 28:1003-1015.
24. **Cover TL and Blaser MJ (1992):** Purification and characterization of the vacuolating toxin from Helicobacter pylori. J Biol Chem., 267:10570–10575
25. **Olfat FO, Zheng Q, Oleastro M et al.(2005):** Correlation of the Helicobacter pylori adherence factor BabA with duodenal ulcer disease in four European countries. FEMS Immunol Med Microbiol., 44:151–156.