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**ORIGINAL ARTICLE**

# Prevalence of *Helicobacter pylori* cagA and vacA Genes and Their Correlation with Gastrointestinal Diseases

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***Key words:***
***H. pylori, Peptic ulcer, Gastrointestinal Diseases, vacA variant and cagA***

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## ABSTRACT

***Background****: Helicobacter pylori (H. pylori) is a gram-negative bacterium, producing gastric ulcer, mild to severe gastritis, gastric carcinoma and lymphoma to the gastric mucosa-associated lymphoid tissue through many virulence influences. Among the virulence factors identified; vacuolating cytotoxin A (vacA) and cytotoxin-associated gene A (cagA) play an important role.* ***Objective:*** *In this study, we try to discover the relation between vacA variant and cagA genes with the clinical illness occurring in H. pylori patients* ***Methodology****: One hundred and forty patients were included in our study. Dual biopsy samples were taken from the stomach; one was examined by the urease test, and the other one was stored at −80°C for DNA extraction followed by PCR. The existence of H. pylori in the tissue was recognized by the existence of glmM gene and its detection by PCR. All the positive samples were additionally tested by PCR for the occurrence of cagA and vacA variant genes.* ***Results:*** *Our study demonstrated that cagA and vacA genes were found among 50% and 57% respectively of H. pylori patients complaining from gastrointestinal illnesses and that vacA s1/s2 was the main genotype found in H. pylori persons with gastroduodenal disease. Significant relation between vacA s1 gene and cagA gene was found.* ***Conclusion:*** *vacA s1 genotype has a vital role in upper gastrointestinal illnesses progress.*

## INTRODUCTION

*Helicobacter pylori (H. pylori)* is one of the gram-negative bacteria, lives in the stomach mucosa in about 50% of the human worldwide, causing different degrees of gastritis, peptic ulcer, gastric cancer and gastric lymphoma[1]**.** Many virulence genes play an essential role in the mechanism of disease formation such as vacuolating cytotoxin A (vacA), cytotoxin-associated gene A (cagA), urease enzyme, flagellae, and adhesins[2]**.**

After, *H. pylori* reaches the stomach, it secretes urease enzyme to counterbalance the gastric HCL. Then, by its flagellae move in the direction of gastric epithelium cells. After that, interactions occur between *H. pylori* adhesins and the gastric cell receptors causing colonization of the stomach tissue. Host tissue damage occurs through the release of multiple toxins, such as vacA and cagA. Moreover, the stomach mucosa releases chemokines which stimulate innate immunity and attract the neutrophils, triggering the progress of medical illnesses such as gastritis and gastric ulcer [3]**.**

*H. pylori* cag pathogenicity island (cag PAI) carries the mechanisms of type IV secretion system (T4SS)" [4]. One of them is cagA. Its production enhances *H. pylori* virulence as cagA stimulates the release of IL-8, the activation of nuclear factor-kB, the occurrence of mucosal inflammation, and lastly the progress to peptic ulcer and gastric carcinoma[5]**.** cagA was known as a cancer-associated agent and is related to the progress to gastric lymphoma [6]**.**

Another virulence factor is vacA, which is existing in approximately 50% of *H. pylori*, and is responsible for the vacuolating cytotoxin effect occuring in many cell lines in vitro. vacA gene comprises two adjustable parts. The s region, which exists as an s1 or s2 allele and is located at the 5' end of the gene (encrypting the signal peptide) and the m-region, which exists as m1 or m2 allele and is located at the middle section of the gene[7].

Some researchers have discovered that peptic ulcer or gastric cancer are more shared in individuals infected with vacA s1 or m1 *H. pylori* variants than in individuals infected with s2 or m2 *H. pylori* variants [8]**.**

Duodenal ulcer promoting gene (dupA) is responsible for ulceration occurring in the duodenum and decrease gastric cancer occurrence in some studies[9]**.** dupA enhances the release of IL-8 and IL-12 from the antrum gastric mucosa both in vivo and in vitro[10]**.** dupA well thought as a disease-specific virulence agent in some nations in East Asian such as South Korea and Japan[11]**.** Also, some studies link the occurrence of dupA was *H. pylori* treatment failure [12]**.**

Researches have been directed to link the clinical illness occurring in *H. pylori* patients with *H. pylori* virulence genes[13]**.** In this study, we try to discover the


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relation between vacA variant and cagA genes and the clinical illness occurring in *H. pylori* patients.

## METHODOLOGY

This study was done in the Genetic Unit of Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Egypt, from January 2019 to February 2020.

**Study Population**

The study deign is a cross-sectional form. One hundred and forty patients were included in the research. They attended the Gastrointestinal Endoscopy Unit at Mansoura University Hospitals, Egypt, complaining of upper GIT symptoms or patients formerly identified as, gastritis, peptic ulcer or carcinoma attending the unit for treatment.

From all patients, complete history was taken ex: age, sex, complain, medications, previous endoscopic findings. Patients received antimicrobial therapy or nonsteroidal anti-inflammatory drugs 2 months preceding to the endoscopy were excluded from our study.

According to the endoscopic results: gastritis, peptic ulcer, and gastric cancer, the patient's number was calculated.

Written complete informed consent was taken from the participants. Approval of the ethics committee was obtained from our Institutional Review Board (IRB).

**Laboratory diagnosis of *H. pylori***

Using an endoscope: two biopsy specimens were taken from the stomach; one was studied by the urease test, and the other biopsy was placed in 1 ml saline solution and kept at −80°C for DNA extraction followed by PCR.

***DNA extraction and PCR amplification***

Extraction of DNA was completed according to the instructions using the extraction kit of QIAmp DNA MINI **(QIAGEN, Germany).**

The existence of *H. pylori* in the tissue was recognized by PCR for glmM gene, which is one of the housekeeping gene. All the positive samples were additionally tested for the occurrence of vacA variants and cagA genes by PCR for individual genes. The corresponding primers and amplicon sizes are revealed in (table 1)**.** *H. Pylori* strain may carry any one of the alternates, for example, VacA s1/m1, VacA s1/m2, VacA s2/m1, or VacA s2/m2.

**Table 1: List of the primers used in the study**

| Gene | Gene primer sequence | Amplicon size (bp) | Reference |
|---|---|---|---|
| **glmM** | F-AAGCTTTTAGGGGTGTTAGGGGTTT<br>R-CGCAATGCTTCAATTCTAAATCTTG | 136 | ***Refaay and Nouh , 2006*** [14] |
| **cagA** | F-GTTGATAACGCTGTCGCTTC<br>R-GGGTTGTATGATATTTTCCATAA | 350 | ***Chattopadhyay et al, 2004*** [15] |
| **vacA s1/s2** | F-ATGGAAATACAACAAACACAC<br>R-CTGCTTGAATGCGCCAAA | 259/286 | ***Chattopadhyay et al, 2004*** [15] |
| **Vac m1/m2** | F-CAATCTGTCCAATCAAGCGAG<br>R-GCGTCAAAATAATTCCAAGG | 567/642 | ***Atherton et al, 1999*** [16] |

Each single PCR reaction mixture was 25 µl and it contains 12.5 µL of master mix (Sigma-Aldrich, India), 1 µl from each primer according to the gene (1 µM, 10 pmol and 25 pmol from the primer of glmM gene , cagA gene and vacA gene respectively) [Sigma-Aldrich, India] and 4 µl of the suspension of DNA. The residual volume was completed with sterile water. Using strain 26695 of *H. pylori* a positive control for *cagA, vacA s1 m1* and *glmM* genes was done and by using sterile water instead of DNA template a negative control was done.

*PCR cycle for glmM gene*: initially, denaturation was done by heating at 95°C for ten min, then 35 cycles for 30 seconds at 95°C, 60°C for 45 seconds; which is the annealing temperature and 72°C for 1 minutes; which is the extension temperature and lastly elongation at 72°C for 5 minutes.[14] While, for *vacA* genes variants and *cagA* were denaturation at 94°C for 3 min, then 35 cycles for 1 minutes at 94°C, 55°C for 1 minutes; which is the annealing temperature and 72°C for 1 minutes; which is the extension temperature and a lastly elongation at 72°C for 10 minutes [15, 16] . Amplified products were visualized by ultraviolet illumination on 1.5% agarose gel electrophoresis containing ethidium bromide. The amplified base pair (bp) length is shown in table 1.

***Statistical Analysis***

Data were analyses using Statistical Set for the Social Sciences software (SPSS version 20, USA, Inc, Chicago, IL). Fisher's exact test and Chi-square test were used to calculate the relation between the genotypes and gastrointestinal findings. For comparison between different groups; Mann–Whitney U test and t-test were used. P value were considered statistically significant if $\leq 0.05$ at confidence interval 95%.

## RESULTS

### *H. pylori* diagnosis

Among 140 patients, 60 patients were infected by *H. pylori* and this was detected by both urease and PCR. PCR was considered positive by the appearance of a band of 294 bp in size on agarose gel, which represent glmM gene (figure 1).

### Virulence genes detection and their relation to the clinical conditions

Recognition of cagA and vacA virulence genes by appearance of their specific bands on agarose gel shows that among the 60 positive *H. pylori* patients: 30 (50%) were cagA positive strains and 34 (57%) were vacA positive strains.

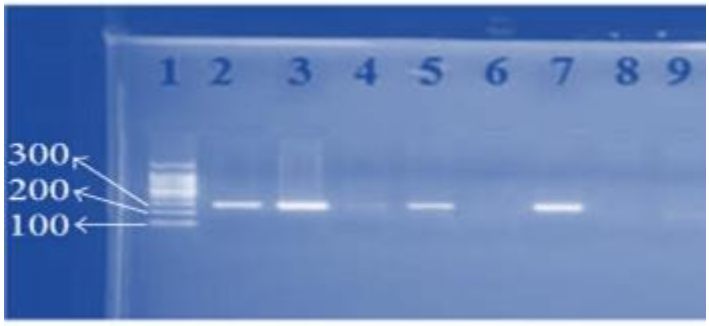


**Fig. 1**: This figure shows the PCR results for *H. pylori* biopsy samples by using glmM gene, which appear as a band of 294 bp in size. Lane 1 is ladders. Lanes 2 to 9 are patients' biopsy specimens.

Our study detected that cagA was present 51.6 % of *H. pylori* patients with gastritis, 44% of *H. pylori* individuals with gastric ulcer, and 75% of *H. pylori* individuals with gastric cancer.

7 isolates contained double vacA genotypes. The existence of gastritis, peptic ulcer and gastric cancer was linked to s1 type strains (P value < 0.05) but not with the m type (P value > 0.05).

Disease occurrence in vacA s1-positive patients had no correlation with the presence or absence of cagA (P value: 0.08).

Among the 31 persons who complained from gastritis, both *s1 m1* and *s1 m2* subtypes represent 59.5%. Peptic ulcer and gastric cancer patients had a similar image in relation to the strains present, as all strains were either *s1 m1* or *s1 m2* variants with no any *vacA s2* variant. Statistical analysis shows a significant P value (less than 0.05) between the *vacA* s1/s2 subtypes and the endoscopic results in *H. pylori* patients (table 2).

**Table 2: Endoscopic findings in *H. pylori* patients and their relation to cag A and vacA genes**

| | Gastritis | | Peptic ulcer | | Gastric cancer | |
|---|---|---|---|---|---|---|
| | N=31 | | N=25 | | N=4 | |
| | N | % | N | % | N | % |
| cag A | 16 | 51.6 | 11 | 44 | 3 | 75 |
| VacA s1/m1 | 11 | 30.5 | 7 | 28 | 2 | 50 |
| VacA s1/m2 | 9 | 29 | 5 | 20 | 0 | 0 |
| VacA s2/m1 | 1 | 3.2 | 0 | 0 | 0 | 0 |
| VacA s2/m2 | 6 | 19.3 | 0 | 0 | 0 | 0 |
| P value between VacA s1 and s2 | 0.01 | | 0.02 | | 0.03 | |
| P value between VacA m1 and m2 | 0.4 | | 0.5 | | 0.1 | |

Co-occurrence of vacA variant and cagA analysis shows that 27 (79.4%) of the vacA s1 isolates were cagA positive, while only 1 (14.3 %) of the vacAs2 isolates was cagA positive.
cagA and vacA s1 were powerfully related (P value: 0.03).

**Table 3: cagA and vacA genes association.**

| | cag A + | cag A - | |
|---|---|---|---|
| vacA s1/m1 | 17 | 3 | P value between cagA and vacA s1: 0.03 |
| vacA s1/m2 | 10 | 4 | |
| vacA s2/m1 | 0 | 1 | P value between cagA and vacA s2: 0.28 |
| vacA s2/m2 | 1 | 5 | |

## DISCUSSION

*Helicobacter pylori* is a dangerous bacterium that inhabits more than half of the world's people, which leads to a great medical load. *H. pylori* diseased individuals have mild to severe gastritis, peptic ulcer and gastric cancer [17].

In this study, diagnosis of *H. pylori* was done by the combination of urease and PCR.

Numerous virulence factors are released from *H. pylori* as urease enzyme, adhesins, vacA and cagA, and are related to gastroduodenal diseases [18].

Among 140 patients, 60 patients were infected by *H. pylori* as approved by both urease and PCR. This study links the endoscopic results of gastrointestinal diseases with certain virulence genes in *H. pylori* as; vacA variant genes and cagA gene and analyzed their existence.

Our study discovered that cagA was existing in 30 (50%) of *H. pylori* patients. However, different researches stated dissimilar percentages of cagA gene in various nations[19]. Studies in Egypt, showed different results as regard the frequency of cagA. Amer and her colleagues informed high rate of cagA gene (65%) among patients tested[20]. Also, Said Essa and his colleagues (2008) stated positive cagA among 62.2% of *H. pylori* infected persons [21]. However, El-Shenawy and his colleagues[22] stated positive cagA gene only among 26.6% of *H. pylori* infected persons. This possibly will be explained by dissimilar socioeconomic grade, sample sizes and living situations of the examined patients [23].

Our study revealed that cagA was found in 51.6 % of *H. pylori* persons with gastritis, 44% of *H. pylori* persons with gastric ulcer, and 75% of *H. pylori* individuals with carcinoma of the stomach.

Some studies found that cagA was more existing in gastric cancer and peptic ulcer than in gastritis [24]. However, Kadi and her colleagues [19] found that cagA gene was more predominant in patients with gastritis than in individuals with peptic ulcer (85% and 77%, *respectively*).

Other studies by Feliciano [25] reported no relation between peptic ulcer and cagA gene. This could be explained by the relatively smaller group of *H. pylori* patients examined in contrast with other studies.

This dissimilarity in cagA percent may be due to alteration of these genes' expression among different countries.

The existence of gastritis, peptic ulcer and gastric cancer was related to vac s1 type strains (P value < 0.05) but not related to the m type (P value > 0.05).

This agrees with Jeyamani et al.[7] , who find that the duodenal ulcer strains in *H. pylori* affected people were vacA s1 m1 type, while, the gastric ulcer strains were 63.6% s1 m1 and 36.4% s1 m2. Also, this study detected that all vacA s1 strain was related to individuals with peptic ulcer.

Jeyamani et al.[7] noted that the *vacA s2* strain is a mild toxin secretor. No one of the patients with peptic ulcer or gastritis had *s2* type. However, *Jeyamani* study found 12 patients with *vacA s2* strain were suffering from gastritis. This possibly will be explained by a milder form of disease development by vacA s2 strains. Their study also showed that *vacA s1* is an important virulence indicator and need therapy.

In our study, the analysis of coincidence of cagA and vacA shows that cagA was positive in 27 (79.4%) of vacA s1 isolates, while only 1 (14.3 %) of vacA s2 isolates was cagA gene positive. This agree *with* Jeyamani et al.[7] who detected that cagA was more frequently related to s1 strain and not related to s2 strain.

## CONCLUSION

Our study showed that cagA and vacA different genes were found among 50% and 57% respectively of *H. pylori* individuals complaining from gastrointestinal diseases.

Positive cagA *H. pylori* strains was correlated with gastritis and gastric ulcer.

vacA s1 genotype has a vital role in upper gastrointestinal illnesses development. So, vacA s1 genotype can be used as a predictor to find individuals who are at a greater risk for progress to gastritis and gastric ulcer. A significant correlation between vacA s1 and cagA genes was found.

To avoid the progress to peptic ulcer in the future, patients diseased with vacA s1 strain should be treated.

**Recommendation:**

We recommend further studies on the other virulence genes of *H. pylori* and its correlation with the clinical conditions.

**Conflicts of interest:**
The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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