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Helicobacter Pylori vacA, *cagA*, *iceA1*, and *iceA2* Genotypes in Gastrointestinal Diseases Patients from Mosul/Iraq

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ABSTRACT

Objective: *Helicobacter pylori is a key cause of gastroduodenal disorders, with cagA-positive strains linked to gastric cancer primarily in Western populations. Other virulent genes may enhance the effects of cagA in disease development. This study sought to identify common H. pylori virulence genes in patients with gastrointestinal disorders.* ***Methods:*** *Biopsy specimens were collected from 100 patients. All DNA samples underwent PCR to confirm H. pylori infection, with positive samples further genotyped. Each PCR reaction used specific primers.* ***Results:*** *EGD findings indicated abnormalities in 89% of patients and normal results in 11%. The gene detection rate was 77%. Analysis of 77 samples showed virulence gene prevalence as 40.3% for cagA, 46.8% for iceA1, and 31.2% for iceA2, with 76.6% containing vacA. The vacA gene was the most common, except in normal findings, where iceA1 was second, followed by cagA and iceA2. High vacA positivity was noted across various conditions, while cagA and iceA2 were absent in some patient groups. A 100% positivity rate for iceA1 was observed in patients with combined gastritis and gastric ulcers. The combination of cagA, iceA1, and vacA was observed in 20.8% of cases, while vacA and iceA1 appeared in 18.2% and vacA and iceA2 in 14.3%. Furthermore, the combination of cagA, iceA2, and vacA was found in 9.1% of cases. Conversely, the combined genotypes of cagA with iceA1, iceA2, and vacA were present at lower frequencies of 2.6%, 3.9%, and 3.9%, respectively. The individual occurrence of virulence factors varied, with vacA present in 10.4% of cases, iceA1 in 5.2%, and iceA2 in 3.9%. Additionally, cagA, iceA1, iceA2, and vacA genes were absent in 7.8% of cases.* ***Novelty:*** *This study reveals unique H. pylori virulence gene combinations and prevalence in Mosul, Iraq, offering regional-specific insights.*

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral-shaped gram-negative bacterium that colonizes the gastric mucosa of more than half of the population worldwide. This bacterium is a leading cause of gastroduodenal diseases. Notably, *H. pylori* has been classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC) since 1994 [1]. The pathogenesis of *H. pylori* infection is associated with their toxins, the most well-studied of which are the virulence factors cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) [2], [3]. The cagA gene, located at the 3'-end of the cag pathogenicity island (cagPAI), encodes the CagA protein, which is prevalent in 60–70% of *H. pylori* worldwide [4], [5]. It is thought that cagA-positive *H. pylori* strains are a risk factor for gastric cancer (GC). However, this appears to be true only for *H. pylori* strains in Western countries, where the cagA-positivity rate for *H. pylori* is only about 40% [6]. At the same time, regardless of gastroduodenal disease, *H. pylori*

strains in East Asian countries exhibit extremely high rates of possessing the cagA gene, up to 90–95% [4], [7], [8]. Therefore, the cagA gene is not the only biological indicator for assessing the clinical outcome caused by *H. pylori* [9]. The vacA gene, which encodes the VacA protein, is found in all *H. pylori* strains. This vacuolating cytotoxin plays an important role in apoptosis and the pro-inflammatory response [7]. The diversity of vacA genotypes causes differences in cytotoxic activity between specific H. pylori strains [10], [11]. The signal region, which has two alleles (s1 and s2), the middle region, which has two alleles (m1 and m2), and the intermediate region, which has two alleles (i1 and i2), are the three main parts of the vacA gene [12], [13]. The *H. pylori* strains with the vacA s1m1 genotype exhibit the highest vacuolating activity, higher than the vacA s2m2 strains [10]. The toxicity of the vacA s1m2 strains is determined by the combination of alleles i1 or i2, with vacA s1i1m2 strains being vacuolating and vacA s1i2m2 strains being non-vacuolating [12].

Although *H. pylori* strains with both the cagA gene and vacuolating toxin-producing vacA genotypes are commonly found in patients with gastroduodenal diseases, a significant number of individuals infected with such *H. pylori* strains remain asymptomatic [14], [15]. Several other *H. pylori* virulence genes have been studied. Peek, in particular, discovered the iceA gene (induced by contact with epithelium gene A), whose transcription was induced by *H. pylori*'s adherence to the gastric epithelium. This gene has two major allelic variants, iceA1 and iceA2 [14]. A number of studies have considered the role of the iceA gene in conjunction with the cagA gene and the vacA gene in the pathogenesis of *H. pylori* [2], [16], [17]. The current study aimed to investigate the prevalence of *H. pylori* cagA, vacA, and iceA and the genotypes in gastrointestinal disease patients. The main objectives of this study were to identify the prevalence and genotypes combination of *H. pylori cagA*, *vacA*, and *iceA* genes among Mosul gastrointestinal disease patients.

RESEARCH METHOD

A. Patients and Clinical Specimens Patients and Clinical Specimens

The study was conducted between April 2023 and May 2024. Gastric biopsies were collected from one hundred patients who attended endoscopic units at Mosul hospitals. At least two gastric mucosa biopsy specimens were obtained from the corpus and antrum. Gastric mucosa biopsy specimens were kept at -20 °C in tubes containing PBS solution until performing the DNA extraction.

B. DNA Extraction

DNA was extracted from all 100 cases to confirm the *H. pylori* positivity and genotyping the *H. pylori cagA, vacA*, and *iceA* genes. First, the biopsy specimens were minced into small pieces using sterile scalpels, and then the DNA was extracted using QIAamp DNA Mini (Qiagen) according to the manufacturer's instructions. DNA concentration and purity were determined by nanodrop.

C. Conventional PCR

All DNA samples extracted from the gastric biopsies were submitted for conventional PCR using primers for 16S rRNA, ureC, and ureA genes to confirm *H. pylori* infection, the positive *H. pylori* for at least one of the three genes were submitted for genotypes using primers for vacA, iceA1, iceA2, and cagA. Each PCR reaction was performed by a single PCR using its specified primers pair except for housekeeping gene *16S rRNA* was semi nested PCR was used. The sequences of primers and amplicon sizes are seen in Table 1.

Table 1. Set of primers used in PCR Technique used in the current study.

Target gene	Primers sequence	Amplicon size(bp)	Annealing temperature °C	Reference
16S rRNA (Semi-nested PCR)	HPl: CTGGAGAGACTAAGCCCTCC Hp3: AGGATGAAGGTTTAAGGATT	446	55	
	HPl: CTGGAGAGACTAAGCCCTCC HP2: ATTACTGACGCTGATTGTGC	110	62	
ureC	glmM-F-AAGCTTTTAGGGGTGTTAGGGGTTT glmM-R-AAGCTTACTTTCTAACACTAACGC	294	55	(18)
ureA	HPU1: GCCAATGGTAAATTAGTT HPU2: CTCCTTAATTGTTTTTAC	411	45	(18)
cagA	F: GATAACAGGCAAGCTTTTGAGG R: CTGCAAAAGATTGTTTGGCAGA	349	52	(10)
iceA1	iceA1F:GTGTTTTTAACCAAAGTATC iceA1R:CTATAGCCASTYTCTTTGCA	247	55	
iceA2	iceA2F:GTTGGGTATATCACAATTTAT iceA2R:TTRCCCTATTTTCTAGTAGGT	229or329	55	(2,16)
vacA	vacA-F F: -GCCGATATGCAAATGAGCCGC vacA-R R: -CAATCGTGTGGGTTCTGGAGC	678	58	(19)

The reactions were carried in 25 µl volumes containing 12.5 GoTaq Green Master Mix 2X (Promega Corp., Madison, WI, USA),1 µl for (10 pmol) each forward and reverse primer, and 3-5 µl (≤ 250 ng/µl) of DNA of the sample extracted DNA, made up to a total volume of 25 µl by adding nuclease-free water. The PCR cycling conditions are illustrated in Table 2. The amplification products were electrophoresed on 2% agarose gel.

Table 2. Optimum conditions were used for the PCR run for all set of primer used in this study to amplify targeted *H. pylori* genes.

Partial amplified gene	Initial denaturation for 5 min.	Cycles N0.	Denaturation for 45 sec.	Annealing for 45 sec.	initial extension for 45 sec.	final extension for 5 min.
16S rRNA	94 °C	35	94 °C	55 °C[1] 62 °C[2]	72 °C	72 °C
ureC	94 °C	35	94 °C	55 °C	72 °C	72 °C
ureA	94 °C	35	94 °C	45 °C	72 °C	72 °C
cagA	94 °C	35	94 °C	55 °C	72 °C	72 °C
vacA	94 °C	35	94 °C	58°C	72 °C	72 °C
iceA1	94 °C	35	94 °C	55 °C	72 °C	72 °C
iceA2	94 °C	35	94 °C	55 °C	72 °C	72 °C

1: annealing temperature for the first round of semi-nested PCR. 2: annealing temperature for the second round of semi-nested PCR.

RESULTS AND DISCUSSION

Results

Specialist physicians reported esopho-gastro-duodenal (EGD) findings. Gastrointestinal disorders of 100 patients were grouped according to EGD findings as having Gastritis (G), Hiatus Hernia (HH), Combined Gastritis and Lax Cardia (GLC), Combined Gastritis and Duodenitis (GD), Combined Gastritis and Hiatus Hernia (GHH), Combined Gastritis and Gastric Ulcers (GGU), and Normal endoscopic finding (N). In Figure 1 the findings from EGD revealed abnormal in (89%) and normal endoscopic in (11%) of the 100 patients.

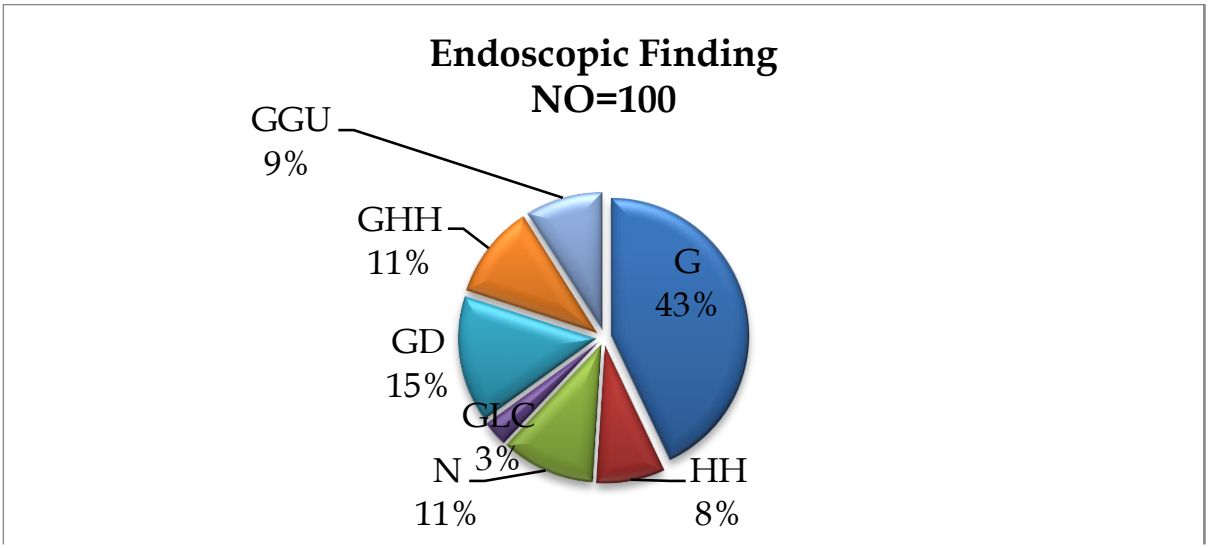

Figure 1. Percentage of endoscopic findings in patients with gastrointestinal disorders.

The detection rate for at least one gene was 77%. Analysis of 77 samples for virulence genes prevalence showed a positive detection of 40.3% for *cagA*, 46.8% for *iceA1*, and 31.2% for *iceA2*. Furthermore,76.6% of samples contained the *vacA* gene. Figure 2 illustrated the distribution of virulence genes among positive gastric biopsy samples for PCR with various endoscopic finding. The *vacA* gene was the predominant virulence gene detected in all endoscopic finding except for patients with normal endoscopic finding, whereas the *iceA1*gene was the second most common gene detected followed by *cagA* gene and finally the least dominant gene, the *iceA2* type. High positive frequency of *vacA* as detected 27/38(71.1%), 4/8(50%), 2/4(50%), 3/3(100%), 9/9(100%), 5/6(83.3%), 9/9(100%) in cases with gastritis, hiatus hernia, normal endoscopic finding, combined gastritis and lax cardia, combined gastritis and duodenitis, combined gastritis and hiatus hernia, combined gastritis and gastric ulcers respectively, while *cagA* and *iceA2* were not detected in patients with hiatus hernia and combined gastritis and gastric ulcers respectively. Also high positive frequency of *iceA1* gene was detected 9/9(100%) in patients with combined gastritis and gastric ulcers.

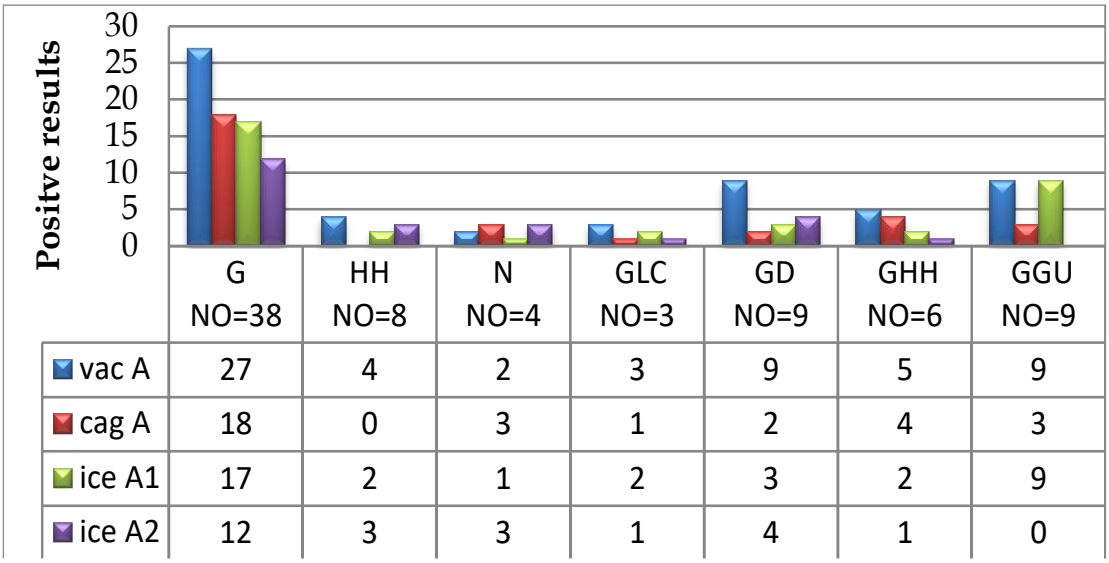

Figure 2. Distribution of virulence genes among positive gastric biopsy samples for PCR with various endoscopic finding.

The genotypes analysis of various *H. pylori* virulence genes revealed significant variations in the frequency of specific combined genotypes associated with virulence factors. The combined genotype of *cagA*, *iceA1*, and *vacA* was detected in 16 out of 77 cases (20.8%), in comparison, the combined genotype of *vacA* and *iceA1* was found in 14 cases (18.2%), while *vacA* and *iceA2* appeared in 11 cases (14.3%). Additionally, the combined genotype of *cagA*, *iceA2*, and *vacA* was detected in 7 cases (9.1%). In contrast, the lower frequencies of the combined genotypes involving *cagA* and *iceA1* (2.6%), *cagA* and *iceA2* (3.9%), and *cagA* and *vacA* (3.9%). The individual presence of the virulence factors also varies, with *vacA* detected alone in 8 out of 77 cases (10.4%), *iceA1* in 4 cases (5.2%), and *iceA2* in 3 cases (3.9%). Moreover, it is notable that virulence genes *cagA*, *iceA1*, *iceA2* and *vacA* were not detected in 6 out of 77 cases (7.8%) as depicted in Table 3.

Table 3. Distribution of various genotype combination of virulence genes among positive gastric biopsy samples for PCR according to endoscopic finding.

Genotype Combination	Endoscopic Finding							
	Gastritis No(%)	Hiatus Hernia No(%)	Normal Endoscopic Finding No(%)	Combined Gastritis and Lax Cardia No(%)	Combined Gastritis and Duodinitis No(%)	Combined Gastritis and Hiatus Hernia No(%)	Combined Gastritis and Gastric Ulcers No(%)	Total No(%)
cagA, iceA1 and *vacA*	11(28.9)	0(0)	0(0)	1(33.3)	0(0)	1(16.7)	3(33.3)	16(20.8)
*cagA, iceA2*and *vacA*	3(7.9)	0(0)	2(50)	0(0)	2(22.2)	0(0)	0(0)	7(9.1)

VacA and iceA1	3(7.9)	1(12.5)	0(0)	1(33.3)	3(33.3)	0(0)	6(66.7)	14(18.2)
vacA and iceA2	4(10.5)	3(37.5)	0(0)	1(33.3)	2(22.2)	1(16.7)	0(0)	11(14.3)
cagA and *iceA1*	1(2.6)	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	2(2.6)
cagA and *iceA2*	3(7.9)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(3.9)
cagA and *vacA*	0(0)	0(0)	0(0)	0(0)	0(0)	3(50)	0(0)	3(3.9)
vacA	6(15.9)	0(0)	0(0)	0(0)	2(22.2)	0(0)	0(0)	8(10.4)
iceA1	2(5.3)	1(12.5)	0(0)	0(0)	0(0)	1(16.7)	0(0)	4(5.2)
iceA2	2(5.3)	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)	3(3.9)
none	3(7.9)	3(37.5)	0(0)	0(0)	0(0)	0(0)	0(0)	6(7.8)
Total	38(100)	8(100)	4(100)	3(100)	9(100)	6(100)	9(100)	77(100)

Discussion

The *cagA*, *vacA*, and *iceA* genotypes are essential for *H. pylori* toxicity. However, the role of these genes in the pathogenesis of gastroduodenal disease is controversial. The main causes are the geographical differences in the prevalence of *H. pylori* virulence genes and genotypes, as well as the synergic interaction between these genes. In this study, we performed DNA extraction from gastric mucosa biopsy specimens in order to investigate *H. pylori* virulence genes. This approach avoided the bacteria culture process, which requires time and labor. Regarding endoscopic findings, our findings aligns with findings from another study conducted in Iraq by [20] reported that (45.2%), (4.3%), (5.7%), (4.8%), (9.5%), (6.2%), (12.4%) and (11.9%) of 210 patients had gastritis, hiatus hernia, lax cardia, normal endoscopic findings, duodenitis, gastric ulcers, duodenal ulcers, and suspected gastric cancer respectively. The detection rates of *cagA* varied across studies, the prevalence ranges from (20.19%) in Burkina Faso [21] to (80%) in Brazil [22] and Vietnam [23]. In Iran, (72%) of *H. pylori*-positive samples contained the *cagA* gene [24], which higher than present study. Whereas in Iraq, a study reporting *cagA* in (39.21%) of specimens [25], moreover in Egypt a study conducted by [26] reported, the *CagA* gene was detected in (42.0%) of *H. pylori* +ve samples, which agree with present study. The *cagA* gene, consider as a marker of pathogenicity, the strains that carry the PAI are known to be more virulent than those lacking it [27]. The *iceA1* and *iceA2* genes are less frequently studied yet in a systematic review and meta-analysis of studies conducted in Iran reported a pooled prevalence of (36.2%) for *iceA1* and (26.2%) for *iceA2*. This suggests a higher prevalence of *iceA1* compared to *iceA2* in Iranian populations, which is consistent with the findings from individual studies in the region [28]. Another study in Iran found that (48%) of gastric biopsies were positive for *iceA1*, while (52%) contained *iceA2*(24). In Turkey, the prevalence rates of *iceA1* and *iceA2* were reported as (58%) and (26%), respectively [29]. A higher prevalence of *iceA1* compared to *iceA2* in this specific population agreed with present study. The *vacA* gene is one of the most prevalent

virulence factors of *H. pylori*. In Iraqi patients, the *vacA* gene was detected in (93.5%) of *H. pylori*-positive samples, making it the most common virulence gene identified [30]. In Iran, the *vacA* gene was present in (68%) of *H. pylori*-positive gastric biopsies, highlighting its widespread occurrence in different populations [24]. The absence of *vacA* in 18 biopsies samples in the current study could be a result of the genetic structure of the strains or exposure to adverse stomach conditions [31], [32]. The presence of *vacA*, along with other virulence factors like *cagA*, enhances the inflammatory response and tissue damage, leading to chronic gastritis and ulceration [33], [34].

CONCLUSION

Fundamental Finding : The study reveals a high prevalence and diversity of Helicobacter pylori virulence genes among individuals with gastrointestinal diseases in Mosul, Iraq. This underscores a significant association between the bacterium's genetic factors and the onset of these health issues, providing critical insights into the pathogenic mechanisms of H. pylori in the region. **Implication :** These findings highlight the urgent need for targeted public health interventions and diagnostic strategies in Mosul to address H. pylori-related diseases. Understanding the genetic diversity of virulence factors can aid in developing tailored treatment protocols and preventive measures to reduce the burden of gastrointestinal disorders. **Limitation :** The research is limited by its geographic focus on Mosul, which may not represent other regions, and by its reliance on a specific population sample. Broader studies incorporating diverse demographics and larger sample sizes are necessary to validate these findings and ensure their generalizability. **Future Research :** Future research should expand to include comparative analyses of H. pylori virulence genes in different regions and populations. Additionally, investigating the role of environmental, dietary, and genetic factors in modulating the bacterium's impact could enhance understanding and inform comprehensive disease management strategies.

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