Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2024; Vol. 22(2)

Association Between *Helicobacter Pylori* Virulence Genes UreA and CagA with Histopathological Changes in Gastroduodenal Disease

Ayat A. Mohammed[1] *MSc*, **Jasim M. Ghadhban**[2] *FICMS (Int Med), CABM, FIBMS (GE&H)*

[1]Dept. of Medical Laboratory Techniques, College of Health and Medical Technology, Ashur University, Baghdad, Iraq,
[2]Dept. of Endoscopy, Gastro-Enterology and Hepatology Teaching Hospital, Medical City Complex, Baghdad, Iraq

Abstract

Background: *Helicobacter pylori* (*H. pylori*) a spiral-shaped bacterium that infects roughly half the world's population, leading to the development of severe complications such as peptic ulcer diseases, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Cytotoxin associated gene A (CagA) and α-subunit of urease (UreA) are two of the major virulence factors of *H. pylori*, and each of them has a distinct entry pathway and pathogenic mechanism during bacterial infection.

Objective: To investigate the relationship of UreA and CagA with the clinical and histopathological finding in patient with different gastro-duodenal diseases.

Methods This is a cross-sectional study included a total of 194 specimens of gastric tissue biopsy (GTB) from patients diagnosed with different gastro-duodenal diseases in period from march 2022 to August 2022. In addition, a histopathologically normal GTBs were obtained from patients. Direct molecular identification of *H. pylori* Ure A and CagA.

Results The results showed that 162/194 (83.5%) of specimens were positive for *H. pylori* UreA and 35/194 (18.0%) of specimens were positive for CagA. The association of positivity to *H. pylori* and oesophagogastroduodenoscopy (OGD) finding was statistically not significant as P-value = 0.079, except for congested mucosa, which was statistically significant as P-value 0.033. The association of positivity to CagA and OGD finding was statistically not significant, P-value = 0.107, except of nodular gastritis which was statistically significant, P-value= 0.001. The association of positivity to cagA with histopathological finding was statistically not significant, P-value= 0.639.

Conclusion The association between UreA and CagA with the OGD and histopathological finding required more investigations. There is association between UreA and congested mucosa, while CagA is associated with nodular gastritis.

Keywords *Helicopacter pylori*, UreA, CagA, conventional PCR

Citation Mohammed AA, Ghadhban JM. Association between *Helicobacter pylori* virulence genes UreA and CagA with histopathological changes in gastroduodenal disease. Iraqi JMS. 2024; 22(2): 259-267. doi: 10.22578/IJMS.22.2.10

List of abbreviations: *H. pylori* = *Helicobacter pylori*, CagA = Cytotoxin-associated gene subunit A, CagPAI = Cytotoxin-associated gene pathogenicity island, GTB = Gastric tissue biopsy, PCR Polymerase chain reaction, UreA = Urease subunit A, UreB = Urease subunit B

Introduction

Helicobacter pylori (*H. pylori*) is a microaerophilic, spiral shaped Gram-negative bacterium that colonizes human stomach [(1)]. It considered the most frequent cause of chronic gastritis and it variably leads to severe gastroduodenal

pathologies in some patients, including gastric and duodenal peptic ulcer disease (PUD), gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [2]. The prevalence of *H. pylori* is high in many countries reporting that 50% of the world's population is infected. The prevalence ranges from 20-50% in industrialized countries to over 80% in developing countries [3]. *H. pylori* had to adapt to the acidic gastric environment by possessing various virulence genes. However, the significance of these virulence genes extends beyond the pure survival needs of the bacteria, making *H. pylori* one of the most well-adapted human pathogens [4]. During infection, *H. pylori* release several of those virulence factors such as urease and cytotoxin-associated gene A (CagA). During *H. pylori* infection, the bacterium can adapt to the acidic gastric environment with the help of urease enzyme. The urease enzyme is made up by a 27-kDa α-subunit (UreA) and a 62-kDa β-subunit (UreB) and can hydrolyze urea to ammonia and bicarbonate [5]. Furthermore, *H. pylori* urease was shown to bind to major histocompatibility complex (MHC) class II molecules and induce cell apoptosis [6]. *H. pylori* strains carrying the cag pathogenicity island (cagPAI) are associated with increased risk of disease progression. The cagPAI encodes the Cag type IV secretion system (CagT4SS), which delivers the CagA oncoprotein and other effector molecules into human gastric epithelial cells [7]. The pathogenicity of CagA protein results in disturbances in cellular signaling, and the expression of CagA increases gastric cancer risk. Infection with cagPAI-positive *H. pylori* strains is considered the strongest risk factor for the development of severe gastroduodenal complications; however, the exact mechanism remains unclear. CagA, after tyrosine phosphorylation, has been demonstrated to cause the cellular pathogenicity seen in *H. pylori i*nfection via different mechanisms [5].

This study aimed to investigate the relationship of UreA and CagA with the clinical and histopathological finding in patient with different gastroduodenal diseases.

Methods

This study is a cross-sectional study, including a total of 194 gastric tissue biopsies (GTBs) collected from patients suffering from gastroduodenal manifestations recruited to Gastroendoscopy Department at Gastroenterology and Hepatology Teaching Hospital, Baghdad, Iraq, from February 2022 to August 2022. According to consultant physician instructions, those patients were subjected to clinical examination and endoscopy. Gastric tissue biopsy samples were taken from the cardia or gastric body part of the stomach (fundus and antrum, respectively). The study was conducted in the microbiology department at Al-Nahrain University-College of Medicine and was approved by the Institutional Review Board of Al-Nahrain University-College of Medicine (IRB/2867/3/2).

DNA was extracted from fresh GTBs using Wizard® Genomic DNA Purification (Cat. No A1120, USA) according to manufacturer instructions. Isolated DNA was stored at -20°C.

Polymerase chain reaction (PCR) was performed using primer set for identification of species-specific UreA in *H. pylori* (F- 5' GCC AAT GGT AAA TTA GTT 3', R- 5' CTC CTT AAT TGT TTT TAC 3') [8]. Briefly, a 25 µL PCR master mix was prepared by adding 1X of PCR Buffer (5X) (Promega/USA), 200 µM of dNTPs (Promega/USA), 30 pMol/reaction (after serial optimizations) of forward and reverse primers (Alpha/Canada) and 1.5 units of Taq DNA polymerase (Promega/USA). Nuclease free H_2O was added to bring the volume to 23 µL. A 2 µL DNA template (100 ng/reaction) was added to the reaction tube, and a no-template control (NTC) tube was prepared with all the PCR master mix components, but with nuclease-free H_2O (2 µL) instead of DNA. The PCR reaction tubes were transferred to a thermal cycler (Eppendorf, Germany) programmed to run at 95°C for 5 min (1X), 35 cycles of 95°C for 1 min, 42°C for 1 min (after 5 serial

optimizations), 72°C for 1 min, and a final extension of 72°C for 7 min. The PCR products were electrophoresed in 1.5% agarose gel. Appearing of band of molecular size 411 bp indicating positivity to *H. pylori*.

PCR was performed using primer set for detection of cag A in *H. pylori* +ve DNA samples (F- 5' GAT AAC AGG CAA GCT TTT GAG G 3', R- 5' CTG CAA AAG ATT GTT TGG CAG A 3') [9]. Briefly, a 50 µL PCR master mix was prepared by adding 1X of PCR Buffer (5X) (Promega/USA), 250 µM of dNTPs (Promega/USA), 20 pMol/reaction (after serial optimizations) of forward and reverse primers (Alpha/Canada) and 3 units of Taq DNA polymerase (Promega/USA). Nuclease-free H_2O was added to bring the volume to 45 µL. A 5 µL DNA template (100 ng/reaction) was added to the reaction tube, and a NTC tube was prepared with all the PCR master mix components, but with nuclease-free H_2O (5 µL) instead of DNA. The PCR reaction tubes were transferred to a thermal cycler (Eppendorf, Germany) programmed to run at 94°C for 10 min (1X), 35 cycles of 94°C for 2 min, 52°C for 3min (after serial optimizations), 72°C for 2 min, and a final extension of 72°C for 10 min. The PCR products were electrophoresed in 1.5% agarose gel. Appearing of band of molecular size 349 bp indicating positivity to Cag A.

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as numbers and percentages. Chi-square test was used to evaluate the association between any two categorical variables; however, Fischer exact test was use instead in cases when the expected count is less than 5 in more 20% of cells. The level of significance was considered at P-value of equal or less than 0.05.

Results

Direct identification of *H. pylori* in 194 extracted DNA from GTBs was done using specific primer set for amplification of UreA gene (Figure 1).

The results showed that 162/194 (83.5%) of extracted DNA samples were positive for *H. pylori*. The association of positivity to *H. pylori* and OGD finding was statistically not significant as P value = 0.079 – 1.000, except for congested mucosa, which was statistically significant as P-value 0.033 as shown in table (1).

The association between positivity to *H. pylori* and histopathological finding of patients from whom GTBs were collected was statistically not significant as the P value = 0.067 (Table 2).

The results showed that 35/194 (18.0%) of extracted DNA were CagA positive (Figure 2) showed agarose gel electrophoresis of PCR amplification results of CagA. The association of positivity to CagA and OGD finding of patients from whom GTBs were collected was statistically not significant, P value = 0.107, except for nodular gastritis which was statistically significant, P value = 0.001 (Table 3).

The association of positivity to CagA with histopathological findings of patients from whom GTBs were collected was statistically not significant, P value = 0.639 (Table 4).

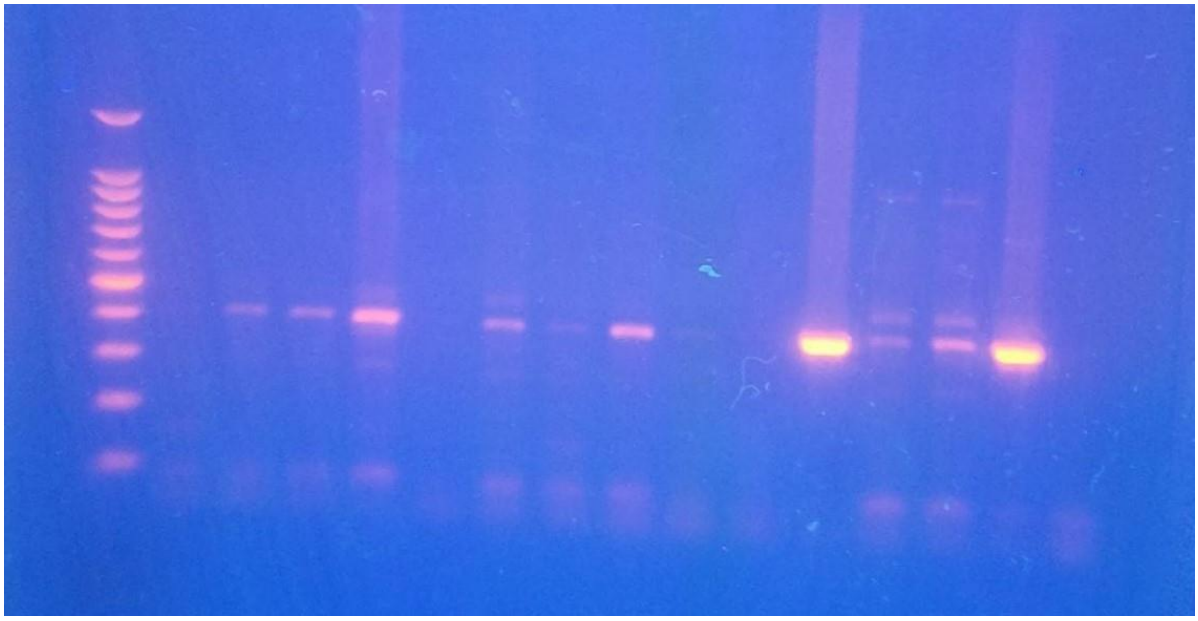

Figure 1. Agarose gel electrophoresis of PCR products of ureA (411bp). Lane (2,3,4,6,7,8,11,12,13,14) amplified sequence of extracted DNA positive to UreA. Lane (1,9): no amplified products of extracted DNA from GTBs of samples (1,9) negative for UreA. Lane (5,10,15): no template control. Lane L: DNA ladder (100bp). Electrophoresis was done in 1.5% agarose gel at (5 V/cm) for 5 hours

Table 1. Molecular identification of *H. pylori* UreA in association with oseophagogastroduodenoscopy findings

OGD findings		*UreA* Positive n = 162 N (%)		*UreA* Negative n = 32 N (%)		P value
Gastritis	Positive	56	34.5	10	31.3	0.717 C
	Negative	106	65.4	2	68.7	NS
Atrophic gastritis	Positive	1	0.6	0	0.0	1.000 F
	Negative	161	99.4	32	100	NS
Nodular gastritis	Positive	14	8.6	1	3.1	0.473 F
	Negative	148	91.4	31	96.9	NS
Mass	Positive	3	1.8	0	0.0	1.000 F
	Negative	159	98.2	32	100	NS
Polyp	Positive	6	3.7	0	0.0	0.592 F
	Negative	156	96.4	32	100	NS
Congestion mucosa	Positive	29	17.9	1	3.1	0.033 F *
	Negative	133	82.1	31	96.9	
Erythematosus mucosa	Positive	16	9.8	0	0.0	0.079 F
	Negative	146	90.2	32	100	NS
Ulcer	Positive	12	7.4	0	0.0	0.222 F
	Negative	150	92.6	32	100	NS
Normal	Positive	19	11.7	5	15.7	0.585 F
	Negative	143	88.3	27	84.3	NS

OGD: Oesophagogastroduodenoscopy; n: Number of cases; C: Chi-square test; F: Fischer exact test; NS: Not significant; *: Significant at $p \leq 0.05$

Table 2. Molecular identification of *H. pylori* UreA in association with histopathological finding

Histopathology findings		*UreA*				P *value*
		Positive n = 28		Negative n = 8		
		N	%	*N*	%	
H. pylori gastritis	Positive	24	85.7	5	62.5	0.167 F
	Negative	4	14.3	3	37.5	NS
Adenocarcinoma	Positive	4	14.3	3	37.5	0.167 F
	Negative	24	85.7	5	62.5	NS

n: Number of cases; F: Fischer exact test; NS: Not significant

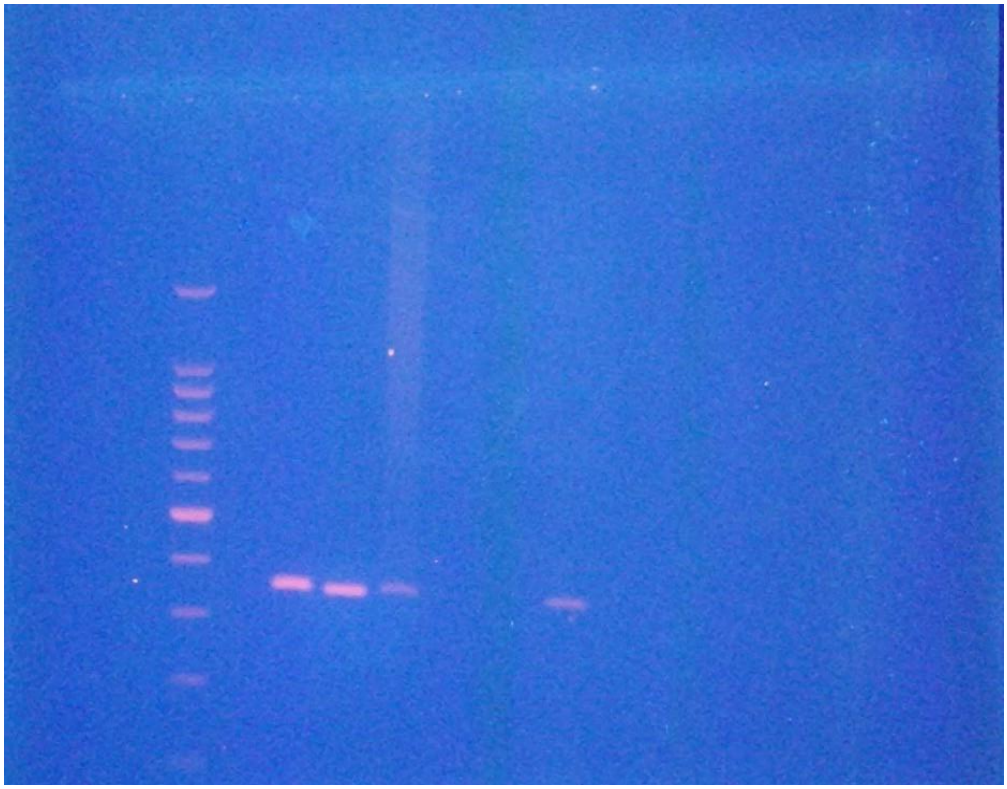

Figure 2. Agarose gel electrophoresis of PCR products of cag A (349bp) Lane (2,3,4,7): positive samples for CagA. Lane (1,6) negative for cagA. Lane (5,8): no template control. Lane L: DNA ladder (100bp). Electrophoresis was done in 1.5% agarose gel at (5 V/cm) for 5 hours

Table 3. Molecular detection of H. pylori CagA in association with oseophagogastroduodenoscopy findings

OGD findings		*CagA* Positive n = 35 N	%	Negative n = 159 N	%	P value
Gastritis	Positive	16	45.7	50	31.4	0.107 C
	Negative	19	54.3	109	68.6	NS
Atrophic gastritis	Positive	0	0.0	1	0.6	1.000 F
	Negative	35	100.0	158	99.4	NS
Nodular gastritis	Positive	8	22.9	7	4.4	0.001 F ***
	Negative	27	77.1	152	95.6	
Mass	Positive	1	2.9	2	1.3	0.451 F
	Negative	34	97.1	157	98.7	NS
Polyp	Positive	2	5.7	4	2.5	0.296 F
	Negative	33	94.3	155	97.5	NS
Congestion mucosa	Positive	5	14.3	25	15.7	0.831 C
	Negative	30	85.7	134	84.3	NS
Erythematosus mucosa	Positive	3	8.6	13	8.2	1.000 F
	Negative	32	91.4	146	91.8	NS
Ulcer	Positive	2	5.7	10	6.3	1.000 F
	Negative	33	94.3	149	93.7	NS
Normal	Positive	2	5.7	22	2	0.260 F
	Negative	33	94.3	137	33	NS

OGD: esophagogastroduodenoscopy; n: number of cases; C: chi-square test; F: Fischer exact test; NS: not significant; ***: significant at p ≤ 0.001

Table 4. Molecular detection of *H. pylori* CagA in association with histopathological history

Histopathology findings		*CagA* Positive n = 28 *N*	%	Negative n = 8 *N*	%	P value
H. pylori gastritis	Positive	6	75.0	23	82.1	0.639 F
	Negative	2	25.0	5	17.9	NS
Adenocarcinoma	Positive	2	25.0	5	17.9	0.639 F
	Negative	6	75.0	23	82.1	NS

n: Number of cases; F: Fischer exact test; NS: Not significant

Discussion

H. pylori is a gram-negative bacterium able to colonize the gastric and duodenal mucosa provoking inflammation and immune response, with pH alteration. It has been related to the appearance of gastric atrophy and intestinal metaplasia, peptic ulcer disease, gastric adenocarcinoma and low-grade malignant B-cell lymphoma [10,11]. The prevalence of *H. pylori* infection is 30-50% in developed countries and 70-90% in developing countries [12]. UreA is housekeeping gene and is required with others genes for urease enzyme activity, which makes this gene valuable in detecting *H. pylori* rather than the 16S rRNA gene even with high sensitivity as this gene is shared by many bacteria [13]. *H. pylori* CagA is a highly immunogenic protein encoded by CagA. It is associated with cell injury and more severe clinical outcomes, including duodenal ulcer and gastric adenocarcinoma [14].

In this study, *H. pylori* was identified directly in extracted DNA from GTBs using specific primers for amplification of UreA and 162/194 (83.5%) samples were positive for *H. pylori*. A study in South Africa (2019) included a total of 444 recruited patients, *H. pylori* was identified in 115 (25.9%) from culture analysis of either antrum or corpus or both biopsies. Two hundred and seventeen patients (48.9%) were positive by the PCR. Ninety-eight (85.2%) of the patients positive for culture were detected in PCR, giving an overall prevalence of 52.7% (i.e. 234/444) [15].

Other study recorded that out of the 50 antrum biopsies screened were positive for *H. pylori* DNA, four types of virulence genes had been detected by PCR. The result showed 16S rRNA had a high percentage of 47 (94%), UreA 46 (92%), UreB 38 (76%), and VacA 11 (22%) [16].

In the current study, the association between positivity to *H. pylori* and OGD finding was significant only with congestion of the mucosa (Mucosal inflammation). The UreA, a gene encoding a protein essential to the survival of *H. pylori*, is regulated via the CrdRS two-component system (TCS) in response to nitric oxide (NO), which is a product of the innate immune system of the human host [17]. *H. pylori* (or its products) may produce gastric inflammation by two main mechanisms; firstly, the organism may interact with epithelial cells on the surface, causing direct cell injury or the release of epithelial-derived pro-inflammatory mediators (chemokines), secondly, *H. pylori*-derived products may obtain access to the mucosa under the surface, triggering non-specific and specific immune responses in the host, including the production of cytokine messengers. Thus, H. pylori infection induces both humoral and cellular immune responses [18].

In the current study, the association between positivity to *H. pylori* and histopathological findings of patients from whom GTBs were collected was statically not significant. As UreA gene, which contribute part for encoding of urease enzyme considered a virulence factor associated with escape to a highly acidic environment not with the advancing of the disease to advance stages [19].

Other study in Sudan, recorded that a total of 290 samples, *H. pylori* were found in 103 samples (35.5%). The highest number of positive *H. pylori* samples were observed in the active chronic gastritis followed by patients of the duodenal ulcer, gastric ulcer, and normal gastric findings in the following frequencies: 75 (25.9%), 13 (4.5%), 6 (2.1%) and 6 (2.1%) respectively, while the lowest frequency was noticed in patients with esophagitis 3 (1.0%) cases [20].

Eighty stomach biopsy samples were isolated in Erbil city, the results of the PCR test based on the UreA gene revealed that 42 (52.5%) of the samples were positive [21].

In the current study, direct molecular detection of CagA in extracted DNA from GTBs samples showed that 35/194 (18.0%) samples were positive.

Iraqi study in 2019 found that 89/104 (85.58%) of *H. pylori* were positive to either CagA, CagE, or both genes [22]. In Saudi Arabia, a study in 2019 found that 63/128 (49.2%) of *H. pylori* identified using real time PCR were positive for CagA [9]. A study in South Africa in 2019 found that 145/234(62%) confirmed H. pylori positive samples were positive for CagA [15].

In the current study, the association between the positivity of *H. pylori* to CagA with OGD findings of patients from whom GTBs were collected was significant only with nodular gastritis. The CagA is not present in all *H. pylori* strains, but is associated with clinical outcomes such as gastritis and PUD as well as a higher risk of occurrence of gastric carcinoma [9]. Nodular gastritis is considered a specific, but insensitive, endoscopic marker for gastric *H. pylori* infection and may associated with the development of diffuse-type gastric cancer [23]. A study in Ukraine in 2023 found that patients with CagA- and VacA-positive *H. pylori* strains had a significant higher grade of inflammation both in the antrum and in the corpus of the stomach and stated that positive CagA and VacA status is related to more severe histopathological changes of gastric mucosa [24].

In the current study, the association between positivity of *H. pylori* CagA with histopathological findings of patients from who GTBs were collected was statically not significant. That may be due to small sample size.

Iranian study in 2023 found that CagA positive H. *pylori* strains augment the risk for severe gastritis, peptic ulceration, atrophic gastritis, dysplasia, and gastric adenocarcinoma compared to strains that lack the cagPAI Cag negative [25].

The CagA, which is one of virulence genes, the risk of infection with CagA positive strains for the development of guanine-cytosine ratio, in not only the prevalence of CagA positive strains but also in the incidence of guanine-cytosine ratio [26].

Various studies in which the *H. pylori* CagA gene alone was transfected into cells have shown that its expression significantly affects the transfected cells. In one report, CagA transfected into human gastric epithelial cells revealed that CagA targets partitioning-defective 1 (PAR1). The CagA–PAR1 interaction causes junctional and polarity defects that release cells from growth-inhibitory signals and promote neoplasia [27].

In conclusion the presence of UreA gene is an indicator for the presence of *H. pylori*. The CagA gene is the major virulence factor associated with nodular gastritis pathology, the presence of this gene can be used as an indicator of the severity of the disease and risk of progression of gastric mucosa.

Acknowledgement
None.

Author contribution
Mohammed: contributed to implementation of the research project and writing the draft of manuscript and contributed to the suggestion of the project idea, interpretation of analytical data, proofreading of research, and writing and producing the research in its final form. Dr. Ghadhban: provided the samples, the clinical diagnosis and physician consultation.

Conflict of interest
The authors declare there is no conflict of interest.

Funding
Self-funding.

References
1. Essawi T, Hammoudeh W, Sabri I, et al. Determination of *Helicobacter pylori* virulence genes in gastric biopsies by PCR. ISRN Gastroenterol. 2013; 2013: 606258. doi: 10.1155/2013/606258.
2. Malfertheiner P, Camargo MC, El-Omar E, et al. Helicobacter pylori infection. Nat Rev Dis Primers. 2023; 9(1): 19. doi: 10.1038/s41572-023-00431-8.
3. Tempera PJ, Michael M, Tageldin O, et al. Gastric cancer due to chronic *H. pylori* infection: what we know and where we are going. Diseases. 2022; 10(3): 57. doi: 10.3390/diseases10030057.
4. Šterbenc A, Jarc E, Poljak M, et al. *Helicobacter pylori* virulence genes. World J Gastroenterol. 2019; 25(33): 4870-84. doi: 10.3748/wjg.v25.i33.4870.
5. Chew Y, Chung HY, Lin PY, et al. Outer membrane vesicle production by *Helicobacter pylori* represents an approach for the delivery of virulence factors CagA, VacA and UreA into human gastric adenocarcinoma (AGS) cells. Int J Mol Sci. 2021; 22(8): 3942. doi: 10.3390/ijms22083942.
6. Fan X, Gunasena H, Cheng Z, et al. *Helicobacter pylori* urease binds to class II MHC on gastric epithelial cells and induces their apoptosis. J Immunol. 2000; 165(4): 1918-24. doi: 10.4049/jimmunol.165.4.1918.
7. Hu B, Khara P, Song L, et al. In situ molecular architecture of the *Helicobacter pylori* Cag type IV secretion system. mBio. 2019; 10(3): e00849-19. doi: 10.1128/mBio.00849-19.
8. Smith SI, Oyedeji KS, Arigbabu AO, et al. Comparison of three PCR methods for detection of *Helicobacter pylori* DNA and detection of cagA gene in gastric biopsy specimens. World J Gastroenterol. 2004; 10(13): 1958-60. doi: 10.3748/wjg.v10.i13.1958.
9. Akeel M, Shehata A, Elhafey A, et al. *Helicobacter pylori* vacA, cagA and iceA genotypes in dyspeptic patients from southwestern region, Saudi Arabia: distribution and association with clinical outcomes and histopathological changes. BMC Gastroenterol. 2019; 19(1): 16. doi: 10.1186/s12876-019-0934-z.
10. Yang F, Zhang J, Wang S, et al. Genomic population structure of *Helicobacter pylori* Shanghai isolates and identification of genomic features uniquely linked with pathogenicity. Virulence. 2021; 12(1): 1258-70. doi: 10.1080/21505594.2021.1920762.
11. Peña-Galo E, Gotor J, Harb Y, et al. Socioeconomic and demographic factors associated with failure in *Helicobacter pylori* eradication using the standard triple therapy. Gastroenterol Hepatol Bed Bench. 2021; 14(1): 53-8.

12. Hussein RA, Al-Ouqaili MTS, Majeed YH. Detection of *Helicobacter Pylori* infection by invasive and non-invasive techniques in patients with gastrointestinal diseases from Iraq: A validation study. PLoS One. 2021; 16(8): e0256393. doi: 10.1371/journal.pone.0256393.

13. AlNaji HA, Omran R, AlSherify A. Molecular detection of *Helicobacter pylori* Infection in gastric biopsy specimens by PCR. Pure Appl Sci. 2018; 26(2): 109-18.

14. Kishk RM, Soliman NM, Anani MM, et al. Genotyping of *Helicobacter pylori* virulence genes cagA and vacA: Regional and national study. Int J Microbiol. 2021; 2021: 5540560. doi: 10.1155/2021/5540560.

15. Idowu A, Mzukwa A, Harrison U, et al. Detection of *Helicobacter pylori* and its virulence genes (cagA, dupA, and vacA) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa. BMC Gastroenterol. 2019; 19(1): 73. doi: 10.1186/s12876-019-0986-0.

16. Sowaid YI, Ali KOM, Abul Hussian SS. Detection of *Helicobacter pylori* by rapid urease test and PCR technique from gastric biopsies. Teikyo Med J. 2022; 45 (1): 4231-46.

17. Allen MG, Bate MY, Tramonte LM, et al. Regulation of *Helicobacter pylori* urease and acetone carboxylase genes by nitric oxide and the CrdRS two-component system. Microbiol Spectr. 2023; 11(1): e0463322. doi: 10.1128/spectrum.04633-22.

18. Kumar S, Patel GK, Ghoshal UC. *Helicobacter pylori*-Induced Inflammation: Possible Factors Modulating the Risk of Gastric Cancer. Pathogens. 2021; 10(9): 1099. doi: 10.3390/pathogens10091099.

19. Ansari S, Yamaoka Y. *Helicobacter pylori* virulence factors exploiting gastric colonization and its pathogenicity. Toxins (Basel). 2019; 11(11): 677. doi: 10.3390/toxins11110677.

20. Elnosh M, Altayb H, Hamedelnil Y, et al. Comparison of invasive histological and molecular methods in the diagnosis of *Helicobacter pylori* from gastric biopsies of Sudanese patients: A cross-sectional study. F1000Res. 2022; 11: 113. doi: 10.12688/f1000research.75873.2.

21. Abdulrahman AT, Ismail SI, Hussain SS, et al. Detection of *Helicobacter Pylori's* virulence gene (UreA) and its Influence on the result of rapid urease test (RUT). Al-Mustansiriyah J Sci. 2022; 33(4): 42-8 . doi: 10.23851/mjs.v33i4.1152.

22. Hamad AL, Shamran HA, Al-Maliki JM, et al. Molecular characteristics and clinical relevance of cytotoxin-associated genes A and E of *Helicobacter pylori* from patients with gastric diseases. Med J Babylon 2019; 16: 156-62. doi: 10.4103/MJBL.MJBL_19_19.

23. Nishikawa I, Kato J, Terasoma S, et al. Nodular gastritis in association with gastric cancer development before and after *Helicobacter pylori* eradication. JGH Open. 2018; 2(3): 80-6. doi: 10.1002/jgh3.12049.

24. Nikulina LM, Solovyova GA, Svintsitskyi IA, et al. Relationship of *Helicobacter pylori* caga and vaca status to morphological changes of gastric mucosa and primary clarithromycin resistance rate in patients with chronic gastritis: A cross-sectional study. Wiad Lek. 2023; 76(4): 709-14. doi: 10.36740/WLek202304103.

25. Azizimoghaddam Y, Kermanpour S, Mirzaei N, et al. Genetic diversity of *Helicobacter pylori* type IV secretion system cagI and cagN genes and their association with clinical diseases. Sci Rep. 2023; 13(1): 10264. doi: 10.1038/s41598-023-37392-7.

26. Sachs G, Weeks DL, Wen Y, et al. Acid acclimation by Helicobacter pylori. Physiology (Bethesda). 2005; 20: 429-38. doi: 10.1152/physiol.00032.2005.

27. Reyes VE. *Helicobacter pylori* and Its Role in Gastric Cancer. Microorganisms. 2023; 11(5): 1312. doi: 10.3390/microorganisms11051312.

Correspondence to Ayat A. Mohammed
E-mail: ayatabbasmohammed@gmail.com

Received Sep. 28th 2023
Accepted Oct. 29th 2023