ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 May 20; 6(5):794-802.
https://doi.org/10.3889/oamjms.2018.178
eISSN: 1857-9655
Clinical Science

Immunopathological and Modulatory Effects of *Cag A*$^{+}$ Genotype on Gastric Mucosa, Inflammatory Response, Pepsinogens, and Gastrin-17 Secretion in Iraqi Patients infected with *H. pylori*

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Citation: Ali Al-Ezzy AI. Immunopathological and Modulatory Effects of *Cag A*$^{+}$ Genotype on Gastric Mucosa; Inflammatory Response; Pepsinogens and Gastrin-17 Secretion in Iraqi Patients infected with *H. pylori*. Open Access Maced J Med Sci. 2018 May 20; 6(5):794-802. https://doi.org/10.3889/oamjms.2018.178

Keywords: pepsinogens; gastrin-17; gastric mucosa; H. pylori; CagA; Iraq

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Received: 04-Feb-2018; **Revised:** 24-Mar-2018; **Accepted:** 25-Mar-2018; **Online first:** 14-May-2018

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

OBJECTIVE: To determine the immunopathological correlation between Cag A+ *H. pylori*-specific IgG; pepsinogen I&II (PI&PII); gastrin-17 (G-17); status of gastric and duodenal mucosa and inflammatory activities on different gastroduodenal disorders.

METHODOLOGY: Eighty gastroduodenal biopsies were taken from patients with gastroduodenal disorders for histopathological evaluation and *H. pylori* diagnosis. Serum samples were used for evaluation of gastric hormones and detection of *H. pylori*-specific IgG antibodies. The tissue expression of *H. pylori Cag A* gene was detected by in situ hybridisation.

RESULTS: *H. pylori* IgG antibodies were detected in (88.8%) of enrolled patients. According to *Cag A* gene expression, Significant difference (P value < 0.05) was detected in levels of PG I; PGII, PG I/PG II among patients with gastric disorders. Serum G-17 level was negatively correlated with *Cag A* gene expression (P-value = 0.04). There was a significant correlation between *H. pylori* IgG and PG I; PG II; G-17. The current study revealed that corpus atrophic gastritis was diagnosed histologically with (5%) gastric ulcer cases; (3.75%) of duodenal ulcer cases; (3.75%) of duodenitis cases; (1.25%) of gastropathy cases and (8.75%) of gastritis cases. At the same time *H. pylori* gastritis diagnosed concurrently with (8.75%) of gastric ulcer cases; (11.25%) of duodenal ulcer cases; (17.5%) of gastropathy cases; (3.75%) of duodenitis cases and (2.5%) of prepyloric ulcer cases. A significant correlation was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding among duodenal ulcer cases and gastritis cases only. A positive correlation was reported between serum levels of PGI; PGII; PGI/PGII; G-17; PMNs grade and Immunopathological status of the gastroduodenal mucosa of *H. pylori* Infected patients. A significant difference was reported in lymphocyte grades among gastric disorders without correlation with immunohistopathological changes in the mucosa (P-value = 0.002). A significant difference was reported in lymphocyte grades among different disorders according to *H. pylori* IgG. A significant difference was reported in serum level of PG I; PG II; PG I/PG II; G-17 according to PMN and lymphocyte grades (P-value < 0.01). PMNs grades positively correlated with gastric Cag A expression; *H. pylori* IgG; PG II; G-17 levels. PG I; PG I/ PG II correlated with lymphocyte grades (P-value < 0.05); while PGII has a negative correlation (P-value = 0.039).

CONCLUSION: Endoscopic mucosal finding does not reflect exactly the actual immunopathological changes of gastric mucosa during *H. pylori* infection. Secretion of gastrin was not affected by the presence of *Cag A* in gastric tissue. Instead, the fluctuation in the hormone level appears to be due to the presence of *H. pylori* infection in gastric tissue. Gastric tissue infiltration with PMNs & lymphocytes inflammatory infiltrates has a direct effect on PGs and gastrin levels in serum of infected patients. The level of PG I; PG II; G-17 secretion correlated with the development of immune response against *H. pylori* and production of specific *H. pylori* IgG. Finally, *H. pylori* can modulate gastric secretions through *Cag A* dependent and independent pathways.

Introduction

There are studies with evidence indicating that Helicobacter pylori play a role in the pathogenesis of various gastroduodenal disorders [1]. *H. pylori* colonisation of gastric tissue induces recruitment of inflammatory cells to the infected gastric epithelium and releasing of virulence factors from the bacteria as opposite reaction [2]. Gastritis induces disruption of acid secretion depending on the predominant location in the stomach, antrum or corpus [3] [4]. The gastroduodenal response to chronic *H. pylori* infection is characterised by infiltration of plasma cells, lymphocytes, neutrophils, and monocytes into gastric mucosa [2]. The gastric epithelium plays an active role

in the mucosal defence. Neutrophil activation and the production of reactive oxygen metabolites are induced directly by bacterial factors and indirectly via host-derived cytokines and products of complement activation [5]. As well as stimulating specific T and B cell responses and systemic immunoglobulin (Ig) G and A antibody production, *H. pylori* infection also induces a local proinflammatory cytokine response and the development of gastric lymphoid follicles which are important in immune cells infiltration [3].

Pepsinogens (PG) are aspartic proteinases, which are mainly secreted by gastric cells. PG can be classified into two biochemically and immunologically distinct types: pepsinogen I (PGI) and pepsinogen II (PGII). PGI is secreted only from the gastric fundic mucosa by chief cells and mucous neck cells in the corpus area [6], while PGII is secreted from the cardiac, fundic, and antral mucosal epithelium of the stomach, and also from the duodenal mucosa [7]. Gastrin-17 is produced mainly by the G cells in the antrum. PGs are released into the circulation and serum PG level reflects the functional and morphologic status of the stomach mucosa. Gastrin-17 (G-17) and pepsinogen I (PGI) levels respectively reflect distal and proximal stomach, while pepsinogen II (PGII) level, reflects the status of the entire stomach and particularly inflammation [8]. Human pepsinogens and gastrin have a diagnostic value for various gastroduodenal disorders, especially for peptic ulcer, atrophic gastritis and gastric cancer [9]. The pepsinogen I/II ratio can provide even better information on the extent of chronic gastritis [10].

The aim of present study was to detect *in situ* expression of *H. pylori Cag A* gene in gastroduodenal biopsies and determination of *H. Pylori*- specific IgG antibodies in serum samples taken from patients presented with gastroduodenal disorders. The second aim was the detection of serum level of gastric hormones (PGI, PGII, PG I/II ratio, G-17) among infected cases. Study the possible correlation between levels of PGI, PGII, PG I/II ratio, G-17, serum H Pylori-IgG antibodies; expression of *Cag A* gene in gastric tissue and status of gastroduodenal mucosa as well as the possible effects of *H. pylori Cag A* gene on levels of gastric hormones and mucosal inflammatory activity.

Subjects and Methods

This cross-sectional, hospital-based study was achieved at gastroenterology department of Baqubah teaching hospital in Diyala province-Iraq after approval of ethical review committee of Department of Pathology, College of Veterinary medicine-Diyala University-Iraq.

A total of, 80 patients presented with clinical indications for upper gastrointestinal tract endoscopy during June 2013 to January 2015 were enrolled. The age range of attended patients (16-80 years) means (47.24 ± 18.82) years. Males represent 44 (55%) versus 36 (45%) females.

This study was conducted according to the principles of Helsinki declaration. A full explanation of the purpose of this study to all patients was done before endoscopy. A signed duly filled consent form obtained from all patients that agree to participate in the study. Exclusion criteria were applied to any patient having a previous gastric surgery; recent or active gastrointestinal bleeding; under antibiotics or colloidal bismuth compounds for past one-month treatment.

Methods

A sterile flexible endoscope was introduced for a full investigation of stomach and duodenum after topical pharyngeal anaesthesia for overnight fasted Patients [4]. Any congested, inflamed or erosive lesions were picked via sterile biopsy forceps. Maximum 6 biopsies were taken. *H. pylori* urease activity was detected in biopsies by placing the samples in Serim® PyloriTek® Test Kit. Each PyloriTek® strip has a built-in positive analyte control and negative control, which run concurrently with the test specimen. The PyloriTek® positive control automatically appears with every test within the normal 1-hour time. With competitive tests, the positive control is run after waiting 24 hours then inserting a urease positive control material [11].

A sterile glass slide with a drop of normal saline was used to teasing the biopsy sample with a sterile scalpel to make smaller fragments of tissue then another sterile glass slide was placed over the teased first tissue, and the tissue was crushed between the two glasses then stain by Gram's staining. The existence of Gram-negative spiral bacteria embedded in the tissue cells was diagnostic for *H. pylori* [12]. true positive results were considered if a combination of urease test and Gram stain give positive results for a single biopsy specimen [13].

In situ hybridisation procedure was used for detection of *H. pylori Cag A* gene expression in 5 µm thickness serial gastric mucosal sections fixed on positively charged slides using biotinylated long DNA probe for H.pylori/ Cag A Gene, Cat. No.: IH-60061(HPY-6001-B) (Maxim biotech-USA) and the DNA Probe hybridisation/Detection System - In Situ Kit (Maxim biotech-USA), according to Maxim biotech instruction manual [14]. The examination and scoring were done under a light microscope by pathologists at powerX400 according to the scoring system [15].

The intensity of gastric inflammation was detected by recording lymphocyte infiltration in gastric tissue via grading scale from 0 to 3, based on both gastric lymphocyte and plasma cell infiltration. Grade

0 considered if normal cellular finding detected. Grade 1 considered in case of low inflammation, Grade 2 for Moderate inflammation and Grade 3 indicate heavy inflammation [16]. Inflammation activity scored as following: None (Grade 0), Rare PMNs(Grade 1); 0-1 intraepithelial (IE) PMN/hpf (Grade 2), Grade (3): 1-10 intraepithelial (IE); PMN/hpf (Grade4): ≥10 IE PMN/hpf [5].

For serological assay; blood was drawn from each patient during the visit to the endoscopy unit. Separated serum samples were stored at 27˚C until analyses. H pylori-specific IgG antibodies were determined using a monoclonal enzyme immunoassay method according to BIOHIT HealthCare instructions [17]. Serum pepsinogen I (PGI) and II (PGII) and gastrin-17 (G-17) were assayed with ELISA using monoclonal antibodies to PGI and II and G-17 (BIOHIT Diagnostics, Biohit, Devon, UK). All procedures were carried out according to the manufacturer's instructions, and results of PGI and II reported in µg/l and pmol/l for gastrin-17. The pepsinogen I: II ratio was calculated and reported in fraction [17].

The frequency of variables expressed as a percentage. PG I, II and G-17 values expressed as mean±standard deviation (Mean±SD). Pearson test for correlation was used for non-categorical data. Chi-test used to compare the PG I, PGII, and G17 according to CagA gene expression. The level of significance was 0.05 (two-tail) in all statistical tests. Significant of correlations(Pearson, Spearman) also include 0.01 (two-tail). Statistical analysis was performed using SPSS for Windows TM version 17.0, and Microsoft EXCEL for windows 2010.

Results

As shown in Table 1, the mean serum level for PGI (112.10 ± 87.73 µg/L) and (40.09 ± 50.80 µg/L) for PGII. Hypersecretion of PGI (> 160µg/L) detected in (31.3%) of patients, mainly among gastropathy; gastritis (8.75%) and duodenal ulcer (DU), (7.5%).

Table 1: Description of Gastric secretions and *H. pylori*-specific serum IgG

Parameters	Minimum	Maximum	Mean±Std. Deviation	Under normal Value	Negative or normal value	Positive or higher than normal
Pepsinogen I (µg/L)	4	400	112.10±87.73	<30 µg/L 6 (7.5%)	30-160 µg/L 49 (61.3%)	> 160 µg/L 25 (31.3%)
Pepsinogen II (µg/L)	6	220	40.09± 50.80	<3 µg/L 0 (0%)	3-15 µg/L 19 (23.8%)	> 15 µg/L 61 (76.3%)
Pepsinogen I / Pepsinogen II ratio	0.17	18.18	4.65± 4.13	<3 µg/L 33 (41.3%)	3-20 µg/L 47 (58.8%)	> 20 µg/L 0 (0%)
Gastrin 17 (pmol/l)	1	400	9.58± 44.30	<1 pmol/ml 0 (0 %)	1-7 pmol/ml 70 (87.5%)	> 7 pmol/ml 10 (12.5%)
H.pylori IgG (EIU)	9.29	250	107.61±52.00	0 (0%)	<30-EIU 9 (11.3%)	>30 EIU 71 (88.8%)

Normal secretion of PGI was detected in gastritis (28.75%) while hyposecretion detected in (3.77%) of gastric ulcer (GU) cases. A significant difference (P-value = 0.005) was detected among gastric disorders in PG I secretion levels as shown in (Table 2).

Hypersecretion of PG II (> 15 µg/L) detected in (76.3%) patients mainly with gastritis (28.75%), gastropathy (16.25%) and DU (15%). The normal value of PG II was detected in (23.8%) of gastric disorders while hyposecretion of PGII not observed with significant difference (P value = 0.006). The mean of PG I/PG II ratio was 4.65 ± 4.13 µg/L. Hyposecretion of PG I/PG II detected in (41.3%), while hypersecretion of PGI/PG II not determined in all gastric disorders with significant difference (P value = 0.000) (Table 2).

The mean of G-17 (9.58 ± 44.30) (pmol/l). Normal range of G-17 (1-7pmol/l) detected in (87.5%) patients; Hypersecretion of G-17 detected in (12.5%) mainly among gastritis (7.5%) without significant difference (P value=0.49) among gastric disorders (Table 2). There was no correlation between serum levels of PG I; PG II; PG I/PG II or G-17 and type of gastroduodenal disorder as shown in Table 2.

As shown in Table 3, the PG I hyposecretion (7.5%), normal (32.5%) and hypersecretion level (18.75%) was significantly higher in CagA positive (P-value = 0.009) (Figure 1).

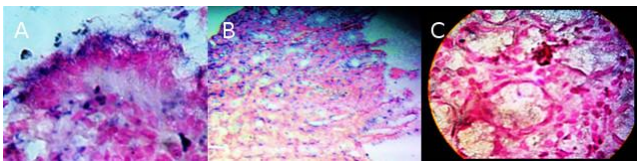

Figure 1: In situ hybridisation for Cag A Positive H. pylori in a gastric tissue section, staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size = 50 µm; A) Gastric epithelia; B) Cag A expression extended to gastric pits; C) negative expression

Significant difference was detected between CagA positive and CagA negative cases in PGII (P value = 0.005); PG I/PG II, (P value = 0.003). No significant difference was detected between patients in G-17 serum level; (P value=0.479). There was no correlation between CagA gene expression and serum levels of PG I; PGII; PG I/PGII but only for Gastrin17 (P value = 0.04). Significant difference and correlation between specific *H. pylori* IgG; PGI (P value = 0.000; P value = 0.004); PG II (P value = 0.000; P value = 0.003); G-17 (P value = 0.000; P value = 0.05). Significant difference without correlation was detected between CagA positive and negative cases in PG I/PG II (P value = 0.000; P value = 0.215) as shown in Table 4.

One of the most interesting points in the current study was that the endoscopic and microscopic examination of gastric mucosa revealed different findings as shown in Table 5.

Table 2: Correlation of Gastric Secretions with Gastroduodenal Disorders

Parameter		Gastric ulcer	Duodenal ulcer	Gastropathy	Gastritis	Duodenitis	Prepyloric ulcer	χ2	P value	r	P value
	<30 µg/L	4 (3.77%)	1 (1.25%)	0 (0%)	0 (0%)	1 (1.25%)	0 (0%)				
Pepsinogen I	30-160 µg/L	8 (10%)	5 (6.25%)	8 (10%)	23 (28.75%)	5 (6.25%)	0 (0%)	157.97	0.005	-0.016	0.887
	> 160 µg/L	3 (3.75%)	6 (7. 5%)	7 (8.75%)	7 (8.75%)	0 (0%)	2 (2.5%)				
	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Pepsinogen II	3-15 µg/L	8 (10%)	0 (0%)	2 (2.5%)	7 (8.75%)	2 (2.5%)	0 (0%)	144.50	0.006	-0.044	0.698
	> 15 µg/L	7 (8.75%)	12 (15%)	13 (16.25%)	23 (28.75%)	4 (3.77%)	2 (2.5%)				
	<3 µg/L	2 (2.5%)	9 (11.25%)	3 (3.75%)	14 (17.5%)	5 (6.25%)	0 (0%)				
Pepsinogen I/ Pepsinogen II ratio	3-20 µg/L	13 (16.25%)	3 (3.75%)	12 (15%)	16 (20%)	1 (1.25%)	2 (1.89%)	266.35	0.000	- 0.054	0.637
	> 20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
	<1 pmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin17	1-7 pmol/l	12 (15%)	12 (15%)	15 (18.75%)	24 (30%)	5 (6.25%)	2 (2.5%)	69.531	0.493	0.075	0.506
	> 7 pmol/l	3 (3.75%)	0 (0%)	0 (0%)	6 (7.5%)	1 (1.25%)	0 (0%)				

Gastric ulcer was diagnosed endoscopically in 15 (18.75%) of patients; 4 (5%) of them have normal gastric mucosa and atrophic corpus gastritis equally, while 7 (8.75%) have H.pylori gastritis without atrophy. A total of 14 (17.5%) have inflamed mucosa during endoscopic examination. Among gastric ulcer cases, no significant correlation was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding (P-value = 0.820).

Table 3: Correlation of Gastric secretions with *Cag A* genotype and *H. pylori*-specific serum IgG

Parameter		CagA positive	CagA Negative	χ2	P value	r	P value
	< 30 µg/L	6 (7. 5%)	0 (0%)				
Pepsinogen I	30-160µg/L	26 (32.5%)	23 (28.75%)	41.900	0.009	0. 085	0.451
	>160 µg/L	15 18.75%)	10 (12.5%)				
	<3 µg/L	0 (0%)	0 (0%)				
Pepsinogen II	3-15 µg/L	11 13.75%)	8 (10%)	41.55	0.005	0. 187	0.097
	>15 µg/L	36 (45%)	25 (31.25%)				
Pepsinogen I/	< 3 µg/L	17 (21.25%)	16 (20%)				
Pepsinogen II	3-20 µg/L	30 (37.5%)	17 (21.25%)	64.52	0.003	0.003	0.980
ratio	>20 µg/L	0 (0%)	0 (0%)				
	< 1 pmol/l	0 (0%)	0 (0%)	13.613	0.479	-0.147	0.194
Gastrin 17	1-7 pmol/l	44 (55%)	26 (32.5%)			-0.229*	0.04*
	>7 pmol/l	3 (3.75%)	7 (8.75%)				

The duodenal ulcer was diagnosed endoscopically in 12 (15%) of patients; 3 (3.75%) of them have atrophic corpus gastritis, while 9 (11.25%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that a total of 9 (11.25%) have normal mucosa during endoscopic examination while 1 (1.25%) suffered from severe erosion and 2 (2.5%) suffered from severe inflammation.

Table 4: Correlation of Gastric secretions with *H. pylori*-specific Serum IgG

Parameter		H. pylori IgG positive	H. pylori IgG Negative	χ2	P value	r	P value
	< 30 µg/L	3 (3.75%)	3 (3.75%)				
Pepsinogen I	30-160 µg/L	44 (55%)	5 (6.25%)	1352.800	0.000	0.317	0.004
	>160 µg/L	24 (30%)	1 (1.25%)				
	<3 µg/L	0 (0%)	0 (0%)				
Pepsinogen II	3-15 µg/L	11 (13.75%)	8 (10%)	1204.127	0.000	0.211	0.06
	>15 µg/L	60 (75%)	1 (1.25%)			0.328*	0.003*
Pepsinogen I/	< 3 µg/L	32 (40%)	1 (1.25%)	1914.333	0.000	0.140	0.215
Pepsinogen II	3-20 µg/L	39 (48.75%)	8 (10%)				
ratio	>20 µg/L	0 (0%)	0 (0%)				
	< 1 pmol/l	0 (0%)	0 (0%)				
Gastrin 17	1-7 pmol/l	65 (81.25%)	5 (6.25%)	593.539	0.000	-0.220	0.05
	>7 pmol/l	6 (7. 5%)	4 (5%)				

* Spearman Correlation.

Among duodenal ulcer cases, a significant difference (P value = 0.027) and the correlation were reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding (P-value = 0.012) as shown in Table 5.

Gastropathy was diagnosed endoscopically in 15 (18.75%) of patients; 1 (1.25%) of them have atrophic corpus gastritis, while 14 (17.5%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that all have inflamed mucosa during endoscopic examination. No statistics are computed because endoscopy mucosal finding is a constant as shown in Table 5.

Gastritis was diagnosed endoscopically in 30 (37%) of patients; 2 (2.5%) of patients have normal mucosa; 7 (8.75%) of them have atrophic corpus gastritis, while 21 (26.25%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that a total of 1 (1.25%) have normal mucosa during endoscopic examination while 3 (3.75%) suffered from severe erosion and 26 (32.5%) suffered from severe inflammation.

Among gastritis cases, a significant difference (P value = 0.001) and the correlation were reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding (P-value = 0.004).

Duodenitis was diagnosed endoscopically in 6 (7.5%) of patients; 3 (3.75%) of them have atrophic corpus gastritis, while 3 (3.75%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that a total of 2 (2.5%) during endoscopic examination suffered from severe erosion and 4 (5%) suffered from severe inflammation. Among duodenitis cases, no significant difference (P value = 0.083) nor correlation (P-value = 0.116) was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding as shown in Table 5. Prepyloric Ulcer was diagnosed endoscopically in 2 (2.5%) of patients; all have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that patients suffered from severe inflammation.

No statistics are computed because endoscopy mucosal finding is a constant. Status of gastroduodenal mucosa significantly differs and correlated with serum levels of PG I (P-value = 0.0000); PG II (P-value = 0.029); PG I/PG II (P-value = 0.008); G-17 (P-value = 0.004) (Table 6). PMNs grades significantly correlated with (P-value = 0.02) status of gastroduodenal mucosa.

Table 5: Correlation of Clinical Diagnosis According To Endoscopy, Immunopathological Status of gastroduodenal mucosa and Endoscopic Mucosal Findings according to *H. pylori* infection

Clinical Diagnosis According To Endoscopy	Immuno-pathological Status of gastroduodenal mucosa	Endoscopy Mucosal Finding			Total	χ^2	P value	r	P value
		Normal	Sever Erosion	Inflammation					
Gastric Ulcer	Normal Mucosa (No Infection)	0 (0%)	0 (0%)	4 (5%)	4 (5%)	2.946	0.229	0.064	0.820
	Atrophic Corpus Gastritis	1 (1.25%)	0 (0%)	3 (3.75%)	4 (5%)				
	H. pylori Gastritis Without Atrophy	0 (0%)	0 (0%)	7 (8.75%)	7 (8.75%)				
	Total	1 (1.25%)	0 (0 %)	14 (17.5 %)	15 (18.75%)				
Duodenal ulcer	Normal Mucosa (No Infection)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7.259	.027	-0.697	0.012
	atrophic corpus gastritis	1 (1.25%)	0 (0%)	2 (2.5%)	3 (3.75%)				
	H. pylori gastritis without atrophy	8 (10%)	1 (1.25%)	0 (0%)	9 (11.25%)				
	Total	9 (11.25%)	1 (1.25%)	2 (2.5%)	12 (15%)				
Gastropathy	Normal Mucosa (No Infection)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	ND	ND*	ND	ND*
	atrophic corpus gastritis	0 (0%)	0 (0%)	1 (1.25%)	1 (1.25%)				
	H. pylori gastritis without atrophy	0 (0%)	0 (0%)	14 (17.5%)	14 (17.5%)				
	Total	0 (0%)	0 (0%)	0 (0%)	15 (18.75%)				
Gastritis	Normal Mucosa (No Infection)	1 (1.25%)	0 (0%)	1 (1.25%)	2 (2.5%)	17.866	.001	0.507	0.004
	atrophic corpus gastritis	0 (0%)	2 (2.5%)	5 (6.25%)	7 (8.75%)				
	H. pylori gastritis without atrophy	0 (0%)	1 (1.25%)	20 (25%)	21 (26.25%)				
	Total	1 (1.25%)	3 (3.75%)	26 (32.5%)	30 (37.5%)				
Duodenitis	Normal Mucosa (No Infection)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3.000	0.083	0.707	0.116
	atrophic corpus gastritis	0 (0%)	2 (2.5%)	1 (1.25%)	3 (3.75%)				
	H. pylori gastritis without atrophy	0 (0%)	0 (0%)	3 (3.75%)	3 (3.75%)				
	Total	0 (0%)	2 (2.5%)	4 (5%)	6 (7.5%)				
Prepyloric Ulcer	Normal Mucosa (No Infection)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	ND	ND*	ND	ND*
	atrophic corpus gastritis	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
	H. pylori gastritis without atrophy	0 (0%)	0 (0%)	2 (2.5%)	2 (2.5%)				
	Total	0 (0%)	0 (0%)	2 (2.5%)	2 (2.5%)				

*No statistics are computed because endoscopy mucosal finding is a constant. ND: not detected.

A significant difference (P-value = 0.002) in grades of mucosal lymphocyte infiltration among gastroduodenal disorders also, there was no correlation between grades of mucosal lymphocyte infiltration and histopathological changes in mucosa as shown in Table 7.

As shown in Table 8, PMNs grades significantly correlated with Cag A expression (P value = 0.0000); *H. pylori* IgG (P value = 0.003). Significant difference in PG I (P value = 0.0000); PG II; PG I/PG II ratio; G-17 according to PMN grade. Significant correlation between PG II (P value = 0.009); G 17 (P value = 0.000) and PMNs Grade.

As shown in Table 9, inflammation intensity according to lymphocyte grade do not correlate with Cag A expression and presence of *H. pylori*-specific IgG (P-value = 0.063), (P-value = 0.706). A significant difference in PG I (P-value = 0.0000); PG II; PG I/PG II ratio; G-17 according to lymphocyte grades; positive correlation between PG I (P-value = 0.007); PG I/PG II (P-value = 0.037) and lymphocyte grades. The negative correlation between PG II (P-value = 0.039) and lymphocyte grades.

Discussion

In this study, the age and gender distribution for *H. pylori*-infected patients come in line with [2] [5] [16] [18] and counteract with recent international studies [19]. *H. pylori* infection provokes both local and systemic antibody responses. The systemic response typically comprises a transient rise in IgM, followed by a rise in specific IgA and IgG maintained throughout infection [20].

In this study, (88.8%) have *H. pylori*-specific IgG antibodies (> 30-EIU) which reflect the high level of immune response to *H. pylori* as the mean of *H. pylori*-specific IgG antibodies (107.61 ± 52) EIU, which come in agreement with [20] and higher than [21] [22], reporting *H. pylori* seropositivity of 56.3%; 57% of Indian and Saudi Arabia patients respectively. The negative *H. pylori*-specific IgG (< 30-EIU) was detected in (11.3%) but, histologically the infection proved through detection of Cag A gene expression in gastric tissue.

Table 6: Correlation Of Gastric Secretions And Status Of Gastroduodenal Mucosa according to *H. pylori* Infection

Parameter		Immunopathological Status of gastroduodenal mucosa			Total	χ^2	P value	Correlation	
		H. pylori Associated atrophic corpus gastritis	*H. pylori* Gastritis without atrophy	Normal mucosa (no infection)				r	P value
Pepsinogen I	< 30 µg/L	6 (7.5%)	0 (0%)	0 (0%)	6 (7.5%)	116.251	0.000	0.408	0.000
	30-160 µg/L	12 (15%)	32 (40%)	5 (6.25%)	49 (61.25%)				
	>160 µg/L	0 (0%)	24 (30%)	1 (1.25 %)	25 (31.25%)				
Pepsinogen II	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	94.710	0.000	0.244	0.029
	3-15 µg/L	5 (6.25%)	9 (11.25%)	5 (6.25%)	19 (23.75%)				
	>15 µg/L	13 (16.25%)	47 (58.75%)	1 (1.25 %)	61 (76.25%)				
Pepsinogen I/ Pepsinogen II ratio	< 3 µg/L	15 (18.75%)	17 (21.25%)	1 (1.25 %)	33 (41.25%)	148.229	0.000	0.095 0.294*	0.403 0.008*
	3-20 µg/L	3 (3.75%)	39 (48.75%)	5 (6.25%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin 17	< 1 pmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	64.856	0.000	-0.317	0.004
	1-7 pmol/l	17 (21.25%)	51 (63.75%)	2 (2.5%)	70 (87.5%)				
	>7 pmol/l	1 (1.25%)	5 (6.25 %)	4 (5%)	10 (12.5%)				

* Spearman Correlation.

Table 7: Correlation of Inflammation intensity; activity and Immunopathological Status of gastroduodenal mucosa according to *H. pylori* Infection

Pmns grade	*Status Of Gastroduodenal Mucosa According To H. pylori Infection*			Total	χ^2	P value	r	P value
	Normal mucosa (No infection)	*H. pylori* corpus Gastritis with atrophy	*H. pylori* gastritis Without atrophy					
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
1	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
2	1 (1.25 %)	3 (3.75%)	2 (2.5%)	6 (7.5%)	6.625	0.157	0.260	0.02
3	3 (3.75%)	9 (11.25%)	21 (26.25%)	33 (41.25%)				
4	2 (2.5%)	6 (7.5%)	33 (41.25%)	41 (51.25%)				
Total	6 (7.5%)	18 (22.5%)	56 (70%)	80 (100%)				
Lymphocyte grade	Normal mucosa (No infection)	Atrophic corpus gastritis	*H. pylori* gastritis without atrophy	Total	χ^2	P value	R	P value
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
1	0 (0%)	2 (2.5%)	0 (0%)	2 (2.5%)				
2	0 (0%)	12 (15%)	24 (30%)	36 (45%)	17.475	0.002	0.037	0.746
3	6 (7.5%)	4 (5%)	32 (40%)	42 (52.5%)				
Total	6 (7.5%)	18 (22.5%)	56 (70%)	80 (100%)				

It means recent infection with a scanty number of *H.pylori* and the time of infection less than 20 IgG seroconversion occurs in 22-23 days after infection [21].

Fluctuations of H. pylori-specific IgG antibody titer predict the variation in response of the host against *H. pylori*. This may give a great possibility for continuous exposure of local population in Iraq to *H. pylori* because of low-quality drinking water, improper sanitation for household sewage, continuous exposure to *H. pylori* from other sources like raw vegetables. All these factors may act in development of the high level of humoral immune response in pre-exposed persons [4].

One of motivating results in the current study was the hypersecretion of PGI (> 160 µg/L), in (31.3%) of patients while hyper secretion of PG II (> 15 µg/L) in (76.3%), both of them among gastropathy; gastritis and duodenal ulcer (P value < 0.05) indicating that the density of the pathogens distributed gradually to be pangastric and even duodenal region stimulating intracellular nitric oxide and calcium production inducing sever inflammatory response due to *H. pylori* that subsequently induce PGs hypersecretion [23] [24]

Cag A expression in gastric tissue appears to play a role in hyposecretion of PGI by fundic gland that was detected in (7.5%) mainly in gastric and duodenal ulcers. All patients infected with Cag A^+ *H. pylori* strain and have a positive association with anti-*H. Pylori* IgG response and histologically gastric and duodenal ulcers associated with corpus atrophic gastritis (table 2, 3 and 4) which explain the main reason for hyposecretion of PGI. Hyposecretion of PGI/PGII detected in (41.3%) among them (21.25%) infected with Cag A^+ *H. pylori* associated with (40%) anti-*H. Pylori* IgG response mainly among Duodenal ulcer, gastritis and duodenitis.

Also, these cases associated with atrophic changes (Tables 2, 3 and 4), the main factor for such disturbance; besides heavy inflammation belongs to PGI because PGII which mainly secreted by pyloric glands and proximal duodenal mucosa still within normal range. These finding supported by others [25] [27] and come in agreement with [26] [28] [29], indicating that serum PG I/PG II ratio decreased when *H. pylori* infection occurs, but the ratio increased after eradication of the bacterium.

In the current study, a normal range of G-17 (1-7 pmol/l) was detected in (87.5%) of patients compared with (12.5%) associated with hypergastrinemia mainly among gastritis (7.5%). A significant correlation (P-value = 0.04) between Cag A expression and serum G-17 level was reported. A negative correlation was recorded between anti-*H. pylori* IgG and serum G17 which come in line with [3] [27]. The current study proved that no correlation between serum levels of PG I; PG II; PG I/PG II or G-17 and type of gastroduodenal disorder that comes in line with [30].

Table 8: Correlation of gastric secretions; inflammatory activity according to PMNs grade; Cag A genotype; *H. pylori*-specific IgG

Parameters		Inflammatory activity according to PMNs grade					Total	χ^2	P value	r	P value
		0	1	2	3	4					
Cag A Genotype	Negative	0 (0%)	0 (0%)	2 (2.5%)	24 (30%)	7 (8.75%)	33 (41.25%)	23.536	0.000	0.381	0.000
	Positive	0 (0%)	0 (0%)	4 (5%)	9 (11.25%)	34 (42.5%)	47 (58.75%)				
H.pylori Igg	<30 EIU	0 (0%)	0 (0%)	4 (5%)	3 (3.75%)	2 (2.5%)	9 (11.25%)	99.232	0.001	0.329	0.003
	>30 EIU	0 (0%)	0 (0%)	2 (2.5%)	30 (37.5%)	39 (48.75%)	71 (88.75%)				
Pepsinogen I	<30 µg/L	0 (0%)	0 (0%)	3 (3.75%)	0 (0%)	3 (3.75%)	6 (7.5%)	90.265	0.000	0.196	0.081
	30-160 µg/L	0 (0%)	0 (0%)	3 (3.75%)	21 (26.25%)	25 (31.25%)	49 (61.25%)				
	>160 µg/L	0 (0%)	0 (0%)	0 (0%)	12 (15%)	13 (16.25%)	25 (31.25%)				
Pepsinogen II	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	92.322	0.000	0.290	0.009
	3-15 µg/L	0 (0%)	0 (0%)	5 (6.25%)	6 (7.5%)	8 (10%)	19 (23.75%)				
	>15 µg/L	0 (0%)	0 (0%)	1 (1.25%)	27 (33.75%)	33 (41.25%)	61 (76.25%)				
Pepsinogen I / Pepsinogen II	<3 µg/L	0 (0%)	0 (0%)	0 (0%)	15 (18.75%)	18 (22.5%)	33 (41.25%)	131.843	0.000	-0.64	0.573
	3-20 µg/L	0 (0%)	0 (0%)	0 (0%)	18 (22.5%)	23 (28.75%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin 17	<1 pmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	40.236	0.05	-0.107 -0.426*	0.347 0.000*
	1-7 pmol/l	0 (0%)	0 (0%)	4 (5%)	25 (31.25%)	41 (51.25%)	70 (87.5%)				
	>7 pmol/l	0 (0%)	0 (0%)	2 (2.5%)	8 (10%)	0 (0%)	10 (30%)				

Table 9: Correlation of gastric secretions; inflammatory intensity according to lymphocytes grade; *Cag A* genotype; *H. pylori*-specific IgG

Parameters		Lymphocyte grade 0	1	2	3	Total	$\chi 2$	P value	r	P value
Cag A In situ	Negative	0 (0%)	2 (2.5%)	17 (21.25%)	14 (17.5%)	33 (41.25%)	4.465	0.107	0.209	0.063
	Positive	0 (0%)	0 (0%)	19 (23.75%)	28 (35%)	47 (58.75%)				
H. Pylori Antibodies	<30 EIU	0 (0%)	0 (0%)	3 (3.75%)	6 (7.5%)	9 (11.25%)	83.222	0.017	-0.043	0.706
	>30 EIU	0 (0%)	2 (2.5%)	33 (41.25%)	36 (45%)	71 (88.75%)				
Pepsinogen I	<30 µg/L	0 (0%)	0 (0%)	6 (7.5%)	0 (0%)	6 (7.5%)	90.860	0.000	0.302	0.007
	30-160 µg/L	0 (0%)	2 (2.5%)	23 (28.75%)	24 (30%)	49 (61.25%)				
	>160 µg/L	0 (0%)	0 (0%)	7 (8.75%)	18 (22.5%)	25 (31.25%)				
Pepsinogen II	>3 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	64.346	0.015	-0.232	0.039
	3-15 µg/L	0 (0%)	0 (0%)	8 (10%)	11 (13.75%)	19 (23.75%)				
	>15 µg/L	0 (0%)	0 (0%)	2 (2.5%)	28 (35%)	31 (38.75%)				
Pepsinogen I/ Pepsinogen II	<3 µg/L	0 (0%)	2 (2.5%)	24 (30%)	7 (8.75%)	33 (41.25%)	154.153	0.000	0.233	0.037
	3-20 µg/L	0 (0%)	0 (0%)	12 (15%)	35 (43.75%)	47 (58.75%)				
	>20 µg/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
Gastrin 17	< 1 pmol/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	50.834	0.005	0.107	0.346
	1-7 pmol/l	0 (0%)	2 (2.5%)	33 (41.25%)	35 (43.75%)	70 (87.5%)				
	>7 pmol/l	0 (0%)	0 (0%)	3 (3.75%)	7 (8.75%)	10 (12.5%)				

The interaction between *H. pylori* and gastrin was shown to be specific, essential and depends on a defined gastrin sequence. In the current study, *H. pylori* cause hypergastrinemia in (12.5%) of infected patients. Enhancement of gastrin secretion in the majority of *H. pylori*-infected patients might be due to several factors. First, increase in leptin production that may be induced after meal or *H. pylori* infection due to direct effect of cholecystokinin (CCK) secretion [30]; reduction of somatostatin secretion as a results of *H. pylori* infection [31] which leads to disruption of the inhibitory effect of somatostatin on the G cell [32]. Mucosal cytokines as a result of *H. pylori* infection, mainly TNFα and IL1β increase gastrin production via G cells [33]. Increased gastrin level reflect the activity of *H. pylori* CagA positive strains in the induction of G cells to increase gastrin mRNA expression in gastric mucosa [31] which give support for present findings that all gastric hormones significantly affected by CagA production in situ [27] [34].

One of the most interesting points in the current study that the endoscopic examination of *H. pylori*-infected gastric mucosa comes with different findings when further assessments take place via histopathology and serological evaluation of GI, GII, G17 (Table 4). This fact reflects the needs for further evaluation of endoscopically *H. pylori* positive cases via histopathological and serological gastric biomarkers for identification of numerous lesions that occurs concurrently in a single patient.

One of the most valuable points in the present study, which revealed by the endoscopic and microscopic examinations of gastric mucosa was different findings. The endoscopic results not exactly reflect the immunopathological changes in gastric tissue. The current study revealed that corpus atrophic gastritis was diagnosed histologically in (5%) gastric ulcer cases; (3.75%) of duodenal ulcer cases; (3.75%) of duodenitis cases; (1.25%) of gastropathy cases and (8.75%) of gastritis cases.

At the same time *H. pylori* gastritis diagnosed concurrently with (8.75%) of gastric ulcer cases; (11.25%) of duodenal ulcer cases; (17.5%) of gastropathy cases; (3.75%) of duodenitis cases and (2.5%) of prepyloric ulcer cases. A significant correlation was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding among gastritis cases in which only (1.25%) has normal mucosa and (3.75%) have severe erosion while (32.5%) suffered from severe inflammation. Among duodenal ulcer cases (11.25%) have normal mucosa during endoscopic examination while (1.25%) suffered from severe erosion and (2.5%) suffered from severe inflammation.

These results come in line with [1] [35] and give assumption of the heavy intensity of Cag A positive (58.75%) *H. pylori* colonization leads to severe inflammatory response and finally to reduction of PGI; PGI/PGII ratio and level of gastrin-17 increased significantly in subjects with atrophic gastritis, which affect the morphology and function of gastric mucosa [3].

A significant correlation was detected between gastric secretions (PGI and PGII; G-17) and status of the gastroduodenal mucosa, whether normal, atrophic or inflamed. Hyposecretion of PGI was reported in (7.5%) of *H. pylori*-associated atrophic corpus gastritis cases, due to the loss of mucosal glands and cells which come in line with [3] [36]. Reasonable hypersecretion of PGI (> 160 µg/L) was detected in (30%) of cases with *H. pylori* mucosal gastritis which may be progressed to ulcers due to hyperchlorhydria [7]. Hypersecretion of PGII was detected in (58.75%) of *H. pylori* mucosal gastritis which gives an obvious indication of pangastric inflammatory pattern compared with (16.25%) in *H. pylori*-associated atrophic corpus gastritis, that may indicate a starting of damage to PGII producing chief cells, which come in accordance with other studies [8]. Hypergastrinemia detected among (6.25%) of *H. pylori*-associated gastritis without atrophy and in (5%) of normal mucosa which explains the role of *H. pylori* infection in limitation of inhibitory activity of D cells producing somatostatin against gastrin production via G cells which come by others [3] [31].

In the current study, a significant correlation (P-value = 0.02) between the status of gastroduodenal

mucosa whether associated with atrophic changes or not and grade of PMNs infiltrated in lamina propria associated with *H. pylori* Infection. No correlation between grades of mucosal lymphocyte infiltration and histopathological changes in mucosa was reported. These results come in line with others, stated that gastric inflammation with *H. pylori* has a considerable impact on the gastric morphology and acid secretion [3]. The present study finding has support from previous studies stated a significant correlation between atrophic changes in the gastric mucosa of Iraqi patients and the activity of lymphocytes and PMNs infiltrated [16].

Reasonable significant correlation between PMNs grades infiltration; specific *H. pylori* IgG and Cag A genotype in situ expression among different disorders were reported, which come by others [5] [16] [25]. The present study reported no correlation (P-value = 0.063) between Cag A in situ expressions; lymphocyte grades infiltration and specific *H. pylori* IgG among different disorders. This may attribute to the fact that numerous virulence factors associated with induction of inflammatory response in infected patients like iceA1, vac A and oip A [37].

A significant difference (P-value = 0.0000) was detected in serum level of PG I; PG II; PG I/PG II ratio; G-17 according to PMNs grade and lymphocyte grades. Significant correlation was detected between PG II (P-value = 0.009); G-17 (P-value = 0.000)and PMNs Grade, which come in agreement with [6] [38], they proved that serum levels of PG II and G 17 increased when gastric mucosa is infiltrated with neutrophils and mononuclear cells in antrum as a result of *H. pylori* infection and its extension into the upper stomach. Others stated that gastrin levels were related to *H. pylori* density and acute/chronic inflammation scores in the corpus mucosa but not in the antral mucosa [39].

The present study recorded a positive correlation between PG I; PG I/PG II ratio and lymphocyte grades infiltrated in lamina propria. While negative correlation was detected between PG II and lymphocyte grades. This finding was supported by previous studies which recorded a correlation between *H. pylori* infection, inflammatory activity in-situ and gastric hormones fluctuation before and after eradication, suggesting that the H. pylori-induced heavy inflammation is a strong stimulus for the synthesis of these biomarkers [6] [40].

In conclusion, the endoscopic mucosal finding does not reflect exactly the actual immunopathological changes of gastric mucosa during *H. pylori* infection. Secretion of gastrin was not affected by the presence of Cag A in gastric tissue. Instead, the fluctuation in the hormone level appears to be due to the presence of H.pylori infection in gastric tissue. Infiltration of gastric tissue with inflammatory infiltrates mainly PMNs and lymphocytes has a direct effect on PGI; PGII and gastrin levels in serum of infected patients. The level of PG I; PG II; G-17 secretion correlated with the development of immune response against *H. pylori* and production of specific *H. pylori* IgG. Finally, *H. pylori* have the ability to modulates gastric secretions through CagA dependent and independent pathways.

Current results recommend the need for further Intensive studies to determine the network of other virulence factors that play a destructive role on the level of gastric hormones and lead to tissue damage that may alter the clinical pathway from simple inflammation to the tumour**.**

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