ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immunopathological Alterations on Gastric Mucosa and Gastric Secretions Induced By Toxigenic *H.pylori* Among Iraqi Patients.

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ABSTRACT

To determine the correlation between toxigenic H.pylori, H.pylori specific IgG; gastric secretions; status of gastric mucosa and inflammatory activity in different gastroduodenal disorders. Gastroduodenal biopsies were taken from patients for histopathology and H.pylori diagnosis. Serum samples were used for evaluation of pepsinogen I (PGI);(PGII); gastrin-17 (G-17) and H pylori specific IgG antibodies. H.pylori Cag A gene expression was detected by *In Situ* Hybridization. H.pylori IgG antibodies detected in (88.8%) .According to Cag A positivity, Significant difference (P value< 0.05) in PG I ;PGII, PG I/ PG II among different gastric disorders . G-17 negatively correlated with Cag A (P value=0.04). Significant correlation between H.pylori IgG and PG I; PG II ; G-17 . Serum levels of PG I; PG II ; PG I / PG II ; G-17 correlated with PMNs grade and Status of H.pylori Infected gastroduodenal mucosa. Lymphocyte grades differs significantly without correlation with histopathological changes in mucosa (P value =0.002) among different disorders according to H.pylori IgG. Significant difference 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 correlated with 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 have negative correlation (P value =0.039) . The level of gastrin secretion does not affected by Cag A expression. The levels of Pepsinogens and gastrin-17 correlated directly with gastric infiltration of PMNs& lymphocytes and serum level of H.pylori IgG. Status of gastroduodenal mucosa significantly correlated with serum levels of pepsinogens and gastrin-17 .H.pylori have the ability to modulate gastric secretions through CagA dependent and independent manner, which subsequently affects the disease progression pattern and final clinical outcome.

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

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ISSN: 0975-8585

INTRODUCTION

Pepsinogens (PG) are aspartic proteinases, which are mainly secreted by gastric cells. PG can be classified in to 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 **[1]**, while PGII is secreted from the cardiac, fundic, and antral mucosal epithelium of the stomach, and also from the duodenal mucosa [2] . Gastrin-17 is produced mainly by the G cells in the antrum. PGs are released in to the circulation and serum PG level reflects the functional and morphologic status of 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[3]. Human pepsinogens and gastrin have a diagnostic value for various gastroduodenal disorders, especially for peptic ulcer, atrophic gastritis and gastric cancer [4] .The pepsinogen I/II ratio can provide even better information on the extent of chronic gastritis [5].

Helicobacter pylori gastric colonization causes recruitment of inflammatory cells by the adherence of pathogen to the epithelium and the release of virulence factors [6] . Gastritis induces disruption of acid secretion depending on the predominant location in the stomach, antrum or corpus [7, 8]. The gastroduodenal response to chronic H. pylori infection is characterized by infiltration of plasma cells, lymphocytes, neutrophils, and monocytes in to gastric mucosa[6].The gastric epithelium plays an active role in mucosal defense. 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[9] . 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 [7].

The aim of present study was to assess the serum PGI, PGII, PG I/II ratio, G-17,serum H Pylori-IgG antibodies and insitu expression of Cag A gene in gastroduodenal biopsies taken from patients presented with gastroduodenal disorders. Study the possible correlation between levels of PGI, PGII, PG I/II ratio, G-17, serum H Pylori-IgG antibodies and insitu expression of Cag A gene 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.

MATERIALS AND METHODS

Patients Selection:

The subjects included in this study consisted of 80 consecutive patients presenting recurrent abdominal pain (range years 16-80, mean age 47.24 ± 18.82) seen at the gastroenterology Clinic, in Baqubah teaching hospital, Diyala province from June 2013 to January 2015. After clinical evaluation, standard laboratory examination, x-ray and ultrasonography, all patients underwent upper-gastrointestinal endoscopy. A written informed consent was obtained from all patients before endoscopy. This study was conducted according to the principles of Helsinki declaration. Approval of ethical review committee of department of pathology, College of Veterinary medicine –Diyala University-Iraq, was taken prior to initiation of the work.

Endoscopy and Biopsies:

Preparation of the patient:

Patients should not have taken antibiotics or bismuth salts for at least three weeks prior to endoscopy. Suppression of H. pylori by these agents makes the organism difficult to detect by any means, and re-growth of H. pylori may be patchy, leading to false negative results in the first few weeks after treatment.

Endoscopy:

Six biopsies were obtained from each patient from the gastric antrum, the corpus and duodenum. One specimen from each site was used for rapid urease test, and two specimens of each were used for

histological analysis. Endoscopic forceps were sterilized in 2% glutaraldehyde solution for a minimum of 20 minutes between experiments.

Detection of urease production and histology

One corpus , one antrum and duodenum sample were used for a rapid urease test employing acutest®, following the manufacturer's instructions, and two corpus and two antrum samples and duodenum samples were used for histopathological examination, including H. pylori detection using the updated Sydney System .

A biopsy specimen for Accutest H. pylori Urease Test may be taken as soon as the endoscopist has examined the stomach. The usual area to biopsy is the sump of the antrum, along the greater curve. Biopsy an area of normal-looking tissue rather than an area affected by erosions or ulceration. This is because H.pylori may be present in smaller numbers if the epithelium is eroded or the mucous layer is denuded. The standard biopsy forceps will provide a specimen of sufficient size (2 - 3 mm diameter).

Accutest h. pylori urease test procedure and interpretation:

The label of the accutest h. pylori urease test was peeled back thus exposing the reactive yellow pad. Immediately after peeling back the label, the specimen from the biopsy forceps was removed using a sterile blunt instrument and placed it onto the reactive yellow pad. The test was re sealed. The label over the reactive pad was pressed lightly with finger to squeeze the tissue contents out of the specimens. On the label, the name of the patient, the date and the time the specimen was inserted.

The Accutest H. pylori urease test was examined at intervals of 5 minutes, 30 minutes and one hour. If any of those intervals or any time in between reveals a positive result, the test is positive. Usually the first attempt to read the accutest h. pylori urease test is made after the endoscopy report has been completed. This allows the endoscopist to objectively report the endoscopic findings before being aware of the presence of H. pylori. If H. pylori are present in the tissue, an expanding red color zone will be noted around the biopsy specimen, or the Accutest H. pylori Urease Test will gradually change to a deep orange, then red color. A red reactive pad anytime within an hour is a positive reaction. A negative result is when the Accutest H. pylori Urease Test reactive pad is still yellow 1 hour after insertion of the specimen.

Insitu hybridization procedure:

H.pylori Cag A gene expression was detected by ISH procedure in 5µm thickness serial gastric mucosal sections fixed on positively charged slides using biotinylated long DNA probe size 349bp, for H.pylori/ Cag A gene, Cat. No. IH-60061(HPY-6001-B) (Maxim biotech-USA) and the DNA Probe hybridization/Detection System – In Situ Kit (Maxim biotech-USA), according to Maxim biotech instruction manual[10] .The examination and scoring were done under light microscope by pathologists at powerX400 according to the scoring system[11].

Scoring of inflammatory infiltrates:

Lymphocyte infiltration, grading scale from 0 to 3, based on both lymphocyte and plasma cell infiltration. Grade 0 considered if normal cellular finding detected. Grade 1 considered if in case of low inflammation, Grade 2 for Moderate inflammation and Grade 3 indicate heavy inflammation[12]. 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) ,grade(4) : ≥ 10 IE PMN /hpf[9] .

Serological assay of H.pylori IgG :

H pylori specific IgG antibodies were determined using a monoclonal enzyme immunoassay method according to Biohit HealthCare instructions [13]

Serological assay of Gastric secretions:

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 .Serum pepsinogen I(PGI) and II(PGII) and gastrin-17 (G-17) were assayed with ELISA using monoclonal antibodies to pepsinogen I and II and gastrin-17 (BIOHIT Diagnostics, Biohit, Devon, UK).All procedures were carried out according to the manufacturer's instructions and results of pepsinogen I and II reported in µg/l and pmol/l for gastrin-17. The pepsinogen I: II ratio was calculated and reported in fraction [13].

Statistical analysis:

Frequency of variables express as percentage. PG I, II and G-17 values express as mean ± standard deviation (Mean ± SD).Pearson test for correlation was used for non-categorical data. Chi-test used to compare the PG PGII, G17, and I according to CagA gene expression. The level of significance was 0.05(two-tail) in all statistical testing; significant of correlations(Pearson, spearman) include also 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 of H.pylori specific IgG antibodies (107.61±52.00) EIU ; (11.3%)of patients have negative H.pylori specific IgG antibodies (<30-EIU) compared with (88.8%) have (>30-EIU). The mean serum level for PGI (112.10±87.73 µg/L) and (40.09± 50.80 µg/L) for PGII.

Hyper secretion of PGI (>160µg/L) detected in (31.3%)of patients ,mainly among gastropathy ; gastritis (8.75%) and duodenal ulcer(DU), (7.5%).Normal secretion of PGI was detected in gastritis (28.75%) while hyposecretion detected in(3.77%) of gastric ulcer(GU) cases. Significant difference (P value= 0.005) was detected among gastric disorders in PG I secretion levels as shown in (Table2).

Hypersecretion of PG II (>15µg/L) detected in (76.3%) patients mainly among gastritis (28.75%), gastropathy (16.25%) and DU (15%). Normal value of PG II was detected in (23.8%) of gastric disorders while hypo secretion of PGII not observed with significant difference (P value= 0.006). The mean of PG I/ PG II ratio (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) (Table2).

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) (Figure1). 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 (3).

One of the most interesting points in current study that the endoscopic and microscopic examination of gastric mucosa comes in different findings as shown in table (4). Corpus atrophy was diagnosed concurrently with (5%)GU; (3.75%) DU; (1.25%) duodenitis; (8.75%), gastropathy and gastritis. H pylori gastritis diagnosed concurrently with (8.75%) GU ;(11.25%) DU; (17.5%) gastropathy; (3.75%) duodenitis; (2.5%) prepyloric ulcer.

Endoscopically normal mucosa represent (13.75%) found to have corpus atrophy in (2.5%) , (10 %) gastritis and only (1.25 %) was actually normal. Severely erosive mucosa accompanied with (5%) of corpus

atrophy. Inflamed gastric mucosa accompanied by corpus atrophy in (15%) and normal mucosa in (6.25%). No significant difference (P value =0.069); nor correlation (P value =0.102) between different gastric disorders in a status of H.pylori infected gastroduodenal mucosa as well as endoscopic mucosal inflammatory findings (P value =0.11), (P value =0.707) as shown in table (4).

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 5). PMNs grades significantly correlated with (P value =0.02) status of gastroduodenal mucosa. 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(6).

As shown in table (7); PMNs grades significantly correlated with Cag A expression (P value =0.0000) ; H.pylori IgG(P value =0.003), while no such correlation reported for lymphocyte grade(P value =0.063) , (P value =0.706). Significant difference in PG I(P value =0.0000); PG II; PG I / PG II ratio ; G-17 according lymphocyte grades and PMN grade. Significant correlation between PG II (P value =0.009); G 17 (P value =0.000) and PMNs Grade. Positive correlation between PG I (P value =0.007); PG I/ PG II (P value =0.037) and lymphocyte grades. Negative correlation between PG II (P value =0.039) and lymphocyte grades .

Table (1): Description of main serological markers under investigation

Parameters	Minimum	Maximum	Mean± SD	under normal Value	normal value	Higher than normal
H.pylori IgG (EIU)	9.29	250	107.61±52.00	0(0%)	<30-EIU 9(11.3%)	>30EIU 71(88.8%)
Pepsinogen I (µg/L)	4	400	112.10±87.73	<30 µg/L 6(7.5%)	30-160µg/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	<1pmol/ml 0(0 %)	1-7pmol/ml 70 (87.5%)	> 7 pmol/ml 10 (12.5%)

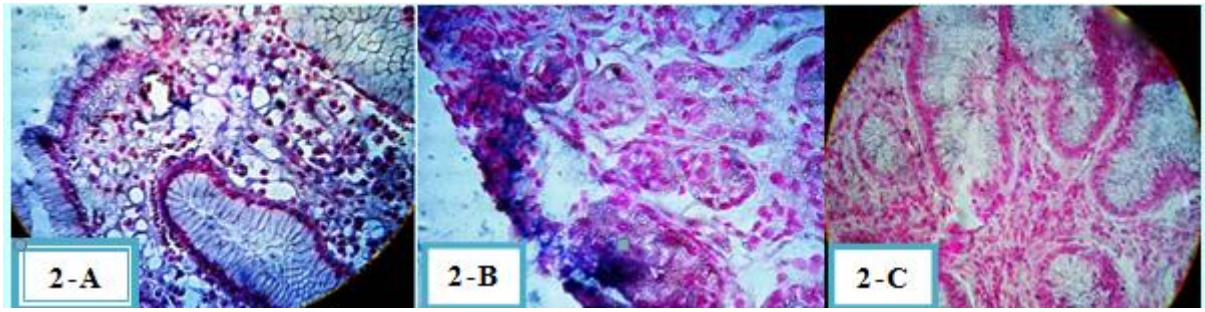

Figure (1): In situ hybridization for CagA Positive H.pylori in gastric tissue section .staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

(A) .Gastric epithelia, (B) CagA expression extended to gastric pits and glands

ISSN: 0975-8585

Table (2): Correlation of gastroduodenal disorders; Pepsinogens and Gastrin17 levels

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

,(C) negative expression

Table (3): Correlation of Pepsinogens and Gastrin17 levels with CagA genotype and H.pylori IgG

Gastric Secretions		CagA positive	CagA Negative	χ2	P value	Pearson Correlation		H.pylori IgG positive	H.pylori IgG Negative	χ2	P value	Pearson Correlation	
						r	P value					r	P value
Pepsinogen I	**< 30µg/L**	**6(7. 5%)**	**0 (0%)**	**41.900**	**0.009**	**0. 085**	**0.451**	**3 (3.75%)**	**3 (3.75%)**	**1352.800**	**0.000**	**0.317**	**0.004**
	30- 160 µg/L	**26 (32.5%)**	**23 (28.75%)**					**44 (55%)**	**5 (6.25%)**				
	>160 µg/L	**15 (18.75%)**	**10(12.5%)**					**24 (30%)**	**1 (1.25%)**				
Pepsinogen II	**<3 µg/L**	**0(0%)**	**0(0%)**	**41.55**	**0.005**	**0. 187**	**0.097**	**0(0%)**	**0(0%)**	**1204.127**	**0.000**	**0.211 0.328***	**0.06 0.003***
	3-15 µg/L	**11 (13.75%)**	**8 (10%)**					**11 (13.75%)**	**8(10%)**				
	>15 µg/L	**36 (45%)**	**25 (31.25%)**					**60 (75%)**	**1 (1.25%)**				
Pepsinogen I/ Pepsinogen II ratio	**< 3µg/L**	**17(21.25%)**	**16(20%)**	**64.52**	**0.003**	**0.003**	**0.980**	**32 (40%)**	**1 (1.25%)**	**1914.333**	**0.000**	**0.140**	**0.215**
	3-20 µg/L	**30(37.5%)**	**17(21.25%)**					**39 (48.75%)**	**8 (10%)**				
	>20 µg/L	**0(0%)**	**0(0%)**					**0(0%)**	**0(0%)**				

ISSN: 0975-8585

Gastrin 17	< 1pmol/l	0(0%)	0(0%)	13.613	0.479	-0.147 -0.229*	0.194 0.04*	0(0%)	0 (0%)	593.539	0.000	-0.220	0.05
	1-7pmol/l	44(55%)	26(32.5%)					65 (81.25%)	5(6.25%)				
	>7pmol/l	3(3.75%)	7(8.75%)					6(7. 5%)	4(5%)				

* Spearman Correlation

Table (4): correlation of Endoscopic parameters and Status of gastroduodenal mucosa

Endoscopic diagnosis	Status of gastroduodenal mucosa according to H.pylori Infection			Total	χ^2	P value	Pearson Correlation	
	normal mucosa (no infection)	H.pylori corpus gastritis with atrophy	H pylori gastritis without atrophy				r	P value
Gastric Ulcer	4(5%)	4(5%)	7(8.75%)	15(18.75%)	17.272	0.069	0.184	0.102
Duodenal Ulcer	0(0%)	3(3.75%)	9(11.25%)	12(15%)				
Gastropathy	0(0%)	1(1.25%)	14(17.5%)	15(18.75%)				
Gastritis	2(2.5%)	7(8.75%)	21(26.25%)	30(37. 5%)				
Duodenitis	0(0%)	3(3.75%)	3(3.75%)	6(7.5%)				
Prepyloric Ulcer	0(0%)	0(0%)	2(2.5%)	2(2.5%)				
Total	6(7.5%)	18(22.5%)	56(70%)	80(100%)				
Endoscopy Mucosal finding	normal mucosa no infection	H.pylori associated atrophic corpus gastritis	H pylori gastritis without atrophy	Total	χ^2	P value	r	P value
normal	1(1.25 %)	2(2.5%)	8(10 %)	11 (13.75%)	7.376	0.117	0.043	0.707
sever erosion	0(0%)	4(5 %)	2(2.5%)	6(7.5%)				
inflammation	5(6.25%)	12(15%)	46(57.5%)	63 (78.75%)				
Total	6(7.5%)	18(22.5%)	56(70%)	80(100%)				

Table (5): correlation of Pepsinogens; Gastrin 17 and Status of gastroduodenal mucosa

Gastric Secretions		Status of gastroduodenal mucosa according to H.pylori Infection			Total	χ^2	P value	Correlation	
		normal mucosa (no infection)	H.pylori corpus gastritis with atrophy	H pylori gastritis without atrophy				r	P value
Pepsinogen I	< 30µg/L	0(0%)	6(7.5%)	0(0%)	6(7.5%)	116.251	0.000	0.408	0.000
	30-160µg/L	5(6.25%)	12(15%)	32(40%)	49(61.25%)				
	>160 µg/L	1(1.25 %)	0(0%)	24(30%)	25(31.25%)				

ISSN: 0975-8585

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%)	5(6.25%)	9(11.25%)	19(23.75%)				
	>15 µg/L	1(1.25 %)	13(16.25%)	47(58.75%)	61(76.25%)				
Pepsinogen I/ Pepsinogen II ratio	< 3µg/L	1(1.25 %)	15(18.75%)	17(21.25%)	33(41.25%)	148.229	0.000	0.095 0.294*	0.403 0.008*
	3-20 µg/L	5(6.25%)	3(3.75%)	39(48.75%)	47(58.75%)				
	>20 µg/L	0(0%)	0(0%)	0(0%)	0(0%)				
Gastrin 17	< 1pmol/l	0(0%)	0(0%)	0(0%)	0(0%)	64.856	0.000	-0.317	0.004
	1-7pmol/l	2(2.5%)	17(21.25%)	51 (63.75%)	70(87.5%)				
	>7pmol/l	4(5%)	1(1.25%)	5(6.25 %)	10 (12.5%)				

* Spearman Correlation

Table (6): correlation of PMNs; Lymphocyte grade and Status of Gastroduodenal mucosa

PMNs grade	*Status of gastroduodenal mucosa according to H.pylori Infection*			Total	χ^2	P value	Correlation	
	normal mucosa (no infection)	H.pylori corpus gastritis with atrophy	H.pylori gastritis without atrophy				r	P value
0	0(0%)	0(0%)	0(0%)	0(0%)	6.625	0.157	0.260	0.02
1	0(0%)	0(0%)	0(0%)	0(0%)				
2	1(1.25 %)	3(3.75%)	2(2.5%)	6(7.5%)				
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 grades	normal mucosa no infection	atrophic corpus gastritis	H.pylori gastritis without atrophy	Total	χ^2	P value	Correlation	
							r	P value
0	0(0%)	0(0%)	0(0%)	0(0%)	17.475	0.002	0.037	0.746
1	0(0%)	2(2.5%)	0(0%)	2(2.5%)				
2	0(0%)	12(15%)	24(30%)	36(45%)				
3	6(7.5%)	4(5%)	32(40%)	42(52.5%)				
Total	6(7.5%)	18(22.5%)	56(70%)	80(100%)				

ISSN: 0975-8585

Table (7): Correlation of inflammatory infiltrates grade; Cag A genotype; H.pylori IgG; Pepsinogens and gastrin 17

Parameters		*PMN grade*					Total	$\chi 2$	*P value*	Correlation	
		0	1	2	3	4				r	P value
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	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
	>30EIU	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
	30-160µg/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	<1pmol/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*
	1-7pmol/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%)				
Parameters		Lymphocyte grade					Total	$\chi 2$	P value	r	P value
		0		1	2	3					
Cag A Genotype	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 IgG	<30-EIU	0(0%)		0(0%)	3(3.75%)	6(7.5%)	9(11.25%)	83.222	0.017	-0.043	0.706
	>30EIU	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
	30-160µg/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
	1-7pmol/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%)				

* Spearman Correlation

ISSN: 0975-8585

DISCUSSION

In this study , the age and gender distribution for H.pylori infected patients come in line with [6, 9, 12, 14] and counteract with recent international studies [15].

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 [16]. 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 [16] and higher than [17, 18], reporting H.pylori seropostivity of 56.3% ;57% of Indian and Saudi Arabia patients respectively . The negative IgG serology(<30-EIU) detected in (11.3%) but histologically the infection proved through detection of Cag A gene expression insitu ,which mean recent infection with a scanty number of H.pylori and the time of infection less than 20 IgG sero conversion occurs in 22-23 days after infection [17].

Fluctuations of H.pylori specific IgG antibody titer predict the variation in an individual's response of the host against H. pylori. This may give idea on continuous exposure of local population in Iraq to this pathogen because of low quality drinking water, improper sanitations for household sewages, continuous exposure to H.pylori from other sources like raw vegetables. All these factors may act in development of high level of humoral immune response in pre-exposed persons.

one of interesting findings in current study was the hypersecretion of PGI(>160µg/L),in (31.3%)of patients while hyper secretion of PG II (>15µg/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 [19, 20]

In current study Cag A expression in gastric tissue appear to play a role in hypo secretion of PGI by fundic gland that detected in (7.5%) mainly in gastric, duodenal ulcers . All patients infected with Cag A strain and have positive association with anti H.pylori IgG response and histologically gastric and duodenal ulcers associated with corpus atrophic gastritis (table2;3&4)which explain the main cause for hypo secretion of PGI . Hypo secretion of PGI/PGII detected in (41.3%) among them (21.25%) infected with Cag A strain associated with (40%) anti H.pylori IgG response mainly among DU, gastritis and duodenitis. Also these cases associated with atrophic changes (table2;3&4) ,the main factor for such disturbance ;beside heavy inflammation belongs to PGI due to the fact that PGII which mainly secreted by pyloric glands and proximal duodenal mucosa still within normal range. These finding supported by others [21-23] . Also these results come in line with others indicating that serum PG I/PG II ratio decrease when H. pylori infection occurs, the, but the ratio increases after the bacterium is eradicated [22, 24, 25] .

In current study normal range of G-17 (1-7pmol/l) was detected in (87.5%) of patients compared with (12.5%) associated with hypergastrinemia mainly among gastritis (7.5%). Significant correlations (P value=0.04) between Cag A expression and serum G-17 level. Inversely anti H.pylori IgG associated with serum G17 which come in line with [7, 23].

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 which come in line with [26] .

The interaction between H.pylori and gastrin was shown to be specific, essential and dependent on defined gastrin sequence. In current study, H.pylori cause alteration in gastrin level among infected patients, hypergastrinemia (12.5%) . Enhancement of gastrin secretion in the majority of H.pylori infected patients in current study might be due to several factors. First , increase in leptin production that may be induced after meal or may be due to H.pylori infection due to direct effect of cholecystokinin (CCK) secretion [26]; reduction of somatostatin secretion as a results of H.pylori infection [27] which leads to disruption of the inhibitory effect of somatostatin on the G cell [28].Mucosal cytokines as a results of H.pylori infection ,mainly TNFα and IL1β increase gastrin production via G cells [29] . Increase in gastrin level reflect the activity of H.pylori CagA positive in induction of G cells to increase gastrin mRNA expression in gastric mucosa[27] which

give a support for present findings that all gastric hormones significantly affected by CagA production insitu [23, 30] .

One of the most interesting points in 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 reflect 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 single patient .

In general, current study revealed that corpus atrophy was diagnosed concurrently with (5%) gastric ulcer; (3.75%) duodenal ulcer; (1.25%) duodenitis; (8.75%),gastropathy and gastritis. H pylori gastritis diagnosed concurrently with (8.75%) gastric ulcers ;(11.25%) Duodenal ulcers; (17.5%) gastropathy; (3.75%) duodenitis; (2.5%) prepyloric ulcer. These results give assumption of the heavy intensity of cag A positive (58.75%)H.pylori colonization that lead to sever 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[7].

In current study, significant correlation was detected between gastric secretions (pepsinogens; Gastrin 17) and status of gastroduodenal mucosa, whether normal, atrophic or inflamed. Hyposecretion of PGI was reported in (7.5%) of H.pylori associated atrophic corpus gastritis due to loss of mucosal glands and cells which come in line with [7, 31] . Reasonable hypersecretion of PGI (>160 µg/L) detected in (30%) of cases with H.pylori mucosal gastritis which may be progressed to ulcers due to hyperchlorhydria [2]. Hypersecretion of PGII was detected in (58.75%) of H.pylori mucosal gastritis that give 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 [3].

Hypergastrinemia detected among (6.25 %) of H pylori associated gastritis without atrophy and in (5%) of normal mucosa which explain the role of H.pylori infection in limitation of inhibitory activity of D cells producing somatostatin against gastrin production via G cells which come in accordance with others [7, 27]

In current study, Significant correlation (P value =0.02) between status of gastroduodenal mucosa whether associated with atrophic changes or not and grade of PMNs infiltrated in lamina propria in associated with H.pylori Infection. No such correlation between grades of mucosal lymphocyte infiltration and histopathological changes in mucosa. 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[7]. Recent finding have support from previous studies stated a significant correlation between atrophic changes in gastric mucosa of Iraqi patients and the activity of lymphocytes and PMNs infiltrated [12].

The current study recorded reasonable significant correlation between PMNs grades infiltration; specific H.pylori IgG and Cag A genotype insitu expression among different disorders, which come in accordance with others [9, 12, 21]. Current study reported no correlation (P value =0.063) between Cag A insitu expression and lymphocyte grade infiltration as well as specific H.pylori IgG among different disorders .this may attributed to the fact that numerous virulence factors associated with induction of inflammatory response in infected patients like iceA1, vac A and oip A[32]

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 PMN grade and lymphocyte grades. Significant correlation was detected between PG II(P value =0.009); Gastrin 17 (P value =0.000)and PMNs Grade. Which come in line with [1, 33] proved that serum level of PG II and G 17 increase when gastric mucosa is infiltrated by 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[34] .

Positive correlation between PG I(P value =0.007); PG I/ PG II ratio (P value =0.037)and lymphocyte grades infiltrated in lamina propria .negative correlation was detected between PG II(P value =0.039) and lymphocyte grades .this finding supported by previous studies which recorded a correlation between H.pylori

infection ,inflammatory activity insitu 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[1, 35].

In conclusion, the level of gastrin 17 secretion does not affected by Cag A expression. The levels of Pepsinogens and gastrin-17 correlated directly with gastric infiltration of PMNs& lymphocytes and serum level of H.pylori IgG. Status of gastroduodenal mucosa significantly correlated with serum levels of pepsinogens and gastrin-17 .H.pylori have the ability to modulate gastric secretions through CagA dependent and independent manner, which subsequently affects the disease progression pattern and final clinical outcome.

REFERENCES

[1] Massarrat S, Haj-Sheykholeslami A, Mohamadkhani A, Zendehdel N, Aliasgari A, Rakhshani N, et al. Pepsinogen II can be a potential surrogate marker of morphological changes in corpus before and after H. pylori eradication. *BioMed research international* 2014; 2014.

[2] Zhang X-m, Li J-x, Zhang G-y, Li X-h, Gu H. The value of serum pepsinogen levels for the diagnosis of gastric diseases in Chinese Han people in midsouth China. *BMC gastroenterology* 2014; 14: 3.

[3] Di Mario F, A; F, Cavallaro L. A Non-invasive Approach to Diagnosis of Upper Gastrointestinal Diseases. Via Comelico, 2008.

[4] Daugule I, Sudraba A, Chiu H-M, Funka K, Ivanauskas A, Janciauskas D, et al. Gastric plasma biomarkers and Operative Link for Gastritis Assessment gastritis stage. *European journal of gastroenterology & hepatology* 2011; 23: 302-7.

[5] Song HJ, Jang SJ, Yun S-C, Park YS, Kim M-J, Lee S-M, et al. Low levels of pepsinogen I and pepsinogen I/II ratio are valuable serologic markers for predicting extensive gastric corpus atrophy in patients undergoing endoscopic mucosectomy. *Gut and liver* 2010; 4: 475-80.

[6] Al-Ezzy AIA. Molecular and Immunopathological role of Gastric versus lymphocytes Interleukin 8 Gene Expression in H.pylori induced Fas-Fasl apoptotic pathway in Gastroduodenal Ulcer in Iraqi patients. *Journal of Biology, Agriculture and Healthcare* 2015; 5: 141-53.

[7] Assoumou MO, Noah D, Ngaba G, Bagnaka SE, Alonge I, Paloheimo I, et al. Association between the immune responses against Helicobacter pylori infections and the concentration of atrophic gastric biomarkers in Cameroon. *Journal of Applied Medical Sciences* 2012; 1: 15-26.

[8] AL-Ezzy AIA. Evaluation of Clinicopathological and Risk Factors for Nonmalignant H. Pylori Associated Gastroduodenal Disorders in Iraqi Patients. *OA Maced J Med Sci* 2015; 3: 655-60.

[9] Al-Ezzy AIA. Molecular and Immunopathological role of nuclear factor k b detected by insitu hybridization in pathogenesis of chronic atrophic gastritis in Iraqi patients. *International Journal of Advanced Research* 2014; 2: 67-78.

[10] Maxim biotech. *Insitu* hybridization detection system

[11] Intisar S. Pity , Baizeed AM. Identification of *Helicobacter pylori* in gastric biopsies of patients with chronic gastritis: Histopathological and immunohistochemical study. . *Duhok Med J* 2011; 5: 69-77.

[12] AL-Ezzy AIA. Immunopathological Role of FAS-FASL Apoptotic Pathway in H. pylori CagA Positive Associated Chronic Atrophic Gastritis in Iraqi Patients. *Journal of Biology, Agriculture and Healthcare* 2014; 4: 67-74.

[13] Biohit H. Biohit gastro panel http://www.biohithealthcare.co.uk/.

[14] AL-Ezzy AIA. In Situ Nick End Labeling as a Molecular Immunopathological Indicator for the Severity of DNA Fragmentationand Gastroduodenal Tissue Damage among H. Pylori Cag APositive Patients. *Indian Journal of Science and Technology* 2016; 9.

[15] Kadhim G, Omar H, Ismail A. Risk Factors Associated with Peptic Ulcer Disease. *J Bioengineer & Biomedical Sci* 2015; 5: 2.

[16] Pandya HB, Patel JS, Agravat HH. Non-Invasive Diagnosis of Helicobacter pylori: Evaluation of Two Enzyme Immunoassays, Testing Serum IgG and IgA Response in the Anand District of Central Gujarat, India. *Journal of clinical and diagnostic research: JCDR* 2014; 8: DC12.

[17] Reddy BS, Venkateswarlu P, Jyothi BN, Devi AR. Role of H. Pylori In Gastroduodenal Diseases. *J of Evolution of Med and Dent Sci* 2015; 4: 581-6.

[18] Sarah Yousef Ahmed, Shammari; HNA. Helicobacter pylori Infection: Seroprevalence and Detection of H. Pylori IgG by Using ELISA. *International Journal of Immunology* 2015; 3: 21-6.

[19] Lorente S, Doiz O, Serrano MT, Castillo J, Lanas A. Helicobacter pylori stimulates pepsinogen secretion from isolated human peptic cells. *Gut* 2002; 50: 13-8.

[20] Lejnieks; MLSLIPDRIVIDABSPJMITA. Pepsinogen testing for evaluation of the success of Helicobacterpylori eradication at 4 weeks after completion of therapy. *m e d i c i n a* 2015; 50: 8-13.

[21] Shiota S, Murakami K, Okimoto T, Kodama M, Yamaoka Y. Serum Helicobacter pylori CagA antibody titer as a useful marker for advanced inflammation in the stomach in Japan. *Journal of gastroenterology and hepatology* 2014; 29: 67-73.

[22] Lu C, Jia H, Xu A, Tang J, Xu G, Yue W, et al. Helicobacter pylori infection and pepsinogen levels have clinical significance in hypertension patients. *International journal of clinical and experimental medicine* 2014; 7: 5675.

[23] Shamsdin SA, Saberifiroozi M, Mehrabani D, Heydari ST. Pepsinogen I and II, Gastrin and Cag A Serum Levels in Shiraz. *Middle East Journal of Digestive Diseases* 2011; 3: 103.

[24] Zhang X, Xue L, Xing L, Wang J, Cui J, Mi J, et al. Low serum pepsinogen I and pepsinogen I/II ratio and Helicobacter pylori infection are associated with increased risk of gastric cancer: 14 year follow up result in a rural Chinese community. *International Journal of Cancer* 2012; 130: 1614-9.

[25] Yanaoka K, Oka M, Ohata H, Yoshimura N, Deguchi H, Mukoubayashi C, et al. Eradication of Helicobacter pylori prevents cancer development in subjects with mild gastric atrophy identified by serum pepsinogen levels. *International Journal of Cancer* 2009; 125: 2697-703.

[26] Honarkar Z, Baladast M, ardakani E, B J, S s. Gastrin, Cholecystokinin (CCK) and H. Pylori in Nonulcer Dyspepsia. *sheraz medical Ejournal* 2007; 8: 13-21.

[27] Amal HS. The relationship between Helicobacter pylori CagA and antral Gastrin (G) and somatostatin (D) cells in chronic gastritis patients *AL-TAQANI* 2010; 23: 135-44.

[28] Liu Y, Vosmaer G, Tytgat G, Xiao S, Ten Kate F. Gastrin (G) cells and somatostatin (D) cells in patients with dyspeptic symptoms: Helicobacter pylori associated and non-associated gastritis. *Journal of clinical pathology* 2005; 58: 927-31.

[29] Huang XQ. Helicobacter pylori infection and gastrointestinal hormones: a review. *World journal of gastroenterology* 2000; 6: 783-8.

[30] Mansour-Ghanaei F, Joukar F, Rajpout Y, Hasandokht T. Screening of Pre-cancerous Gastric Lesions by Serum Pepsinogen, Gastrin-17, Anti-Helicobacter Pylori and Anti-Caga Antibodies in Dyspeptic Patients over 50 years Old in Guilan Province, North of Iran. *Asian Pac J Cancer Prey* 2014; 15: 7635-8.

[31] Pasechnikov Victor D. ZSC, M. Sergey Kotelevets, N. Alexander Mostovov, P. Varvara Mernova and Maria Polyakova,. Invasive and Non-invasive diagnosis of Helicobacter pylori associated atrophic gastritis: A comparative study, . *Scand J Gastroenterol* 2005; 40: 297-301.

[32] Salimzadeh L, Bagheri N, Zamanzad B, Azadegan-Dehkordi F, Rahimian G, Hashemzadeh-Chaleshtori M, et al. Frequency of virulence factors in Helicobacter pylori-infected patients with gastritis. *Microbial Pathogenesis* 2015; 80: 67-72.

[33] Haj sheykholeslami A, Rakhshani N, Amirzargar A, Rafiee R, Shahidi SM, Nikbin B, et al. Serum pepsinogen I, pepsinogen II, and gastrin 17 in relatives of gastric cancer patients: comparative study with type and severity of gastritis. *Clinical Gastroenterology and Hepatology* 2008; 6: 174-9.

[34] Chuang C, Sheu B, Yang H, Kao A, Cheng H, Yao W. Hypergastrinemia after *Helicobacter pylori* infection is associated with bacterial load and related inflammation of the oxyntic corpus mucosa. *Journal of Gastroenterology and Hepatology* 2004; 19: 988–93.

[35] Pimanov SI, Makarenko EV, Voropaeva AV, Matveenko ME, Voropaev EV. Helicobacter pylori eradication improves gastric histology and decreases serum gastrin, pepsinogen I and pepsinogen II levels in patients with duodenal ulcer. *Journal of gastroenterology and hepatology* 2008; 23: 1666-71.