The relationship between *Helicobacter pylori* CagA and antral Gastrin (G) and somatostatin (D) cells in chronic gastritis patients[+]

[*] **Amal H. Salman**

العلاقة بين *Helicobacter pylori* CagA و إفراز الكاسترين(ج) والسوماتوستاتين (د) في مرضى التهاب المعدة المزمن.

المستخلص:

الهدف من هذه الدراسة هو إيجاد العلاقة بين مستويات التعبير في الموضع لجين *Helicobacter pylori* CagAعلى عدد خلايا الكاسترين (ج) والسوماتوستاتين(د) في نسيج المعدة والتي تم الكشف عنها بالتحليل الكيميائي النسيجي المناعي.

طريقة الدراسة: تم التحري عن تعبير جين *H.pylori* CagA في المقاطع النسيجية المطمورة بالبرافين للخزع النسيجية المأخوذة من المعدة وذلك باستخدام تقنية التهجين في الموضع باستعمال مسبار خاص بجين CagA كذلك تم التحري عن تعبير خلايا الكاسترين (ج) والسوماتوستاتين(د) وذلك باستخدام التحليل الكيميائي النسيجي المناعي وباستعمال أضداد وحيدة النسلية.

النتائج: وجد ان نسبة عدد خلايا (ج) تزداد معنويا في المرضى المصابين ببكتيريا *H.pylori* اذا ما تم مقارنتها مع المرضى غيرا لمصابين ببكتيريا *H.pylori* والاشخاص الاصحاء($p<0.01$). وعدد خلايا (د) تزداد معنويا في المرضى الغير المصابين بالبكتريا والأشخاص الأصحاء مقارنة مع المرضى المصابين بالبكتيريا. ووجد إن جين CagA يشارك في ارتفاع نسبة خلايا (ج) بينما ليس له تأثير على خلايا (د).

الاستنتاج: تبين الدراسة إن الاصابة ببكتيريا *H.pylori* تزيد تعبير خلايا (ج) و تختزل تعبير خلايا (د) وان *H.pylori* الموجبة لجين CagA قد تلعب دورا مهما في ازدياد تعبير خلايا (ج) ولكن ليس لها تأثيرا على تعبير خلايا (د) وفي النتيجة سوف يصبح هبوط في وظيفة خلايا (د) مقارنة مع خلايا (ج) التي ربما تكون احد أسباب زيادة نسبة الكاسترين في المعدة وبالتالي في الدم.

Abstract:

Objectives: Investigate the relationship between in situ expression of *Helicobacter pylori* CagA and the numbers of gastrin (G-cells) and somatostatin (D-cells) in gastric tissue investigated by immunohistochemical analysis. **Methods:** The paraffin embedded sections for antral mucosal biopsy specimens were examined using in situ hybridization technique with probe specific for CagA and investigated the expression of gastrin (G-cells) and somatostatin (D-cells) proteins by immunohistochemistry using specific monoclonal antibodies. **Results:** The number of G-cells per gastric gland was significantly higher in patients infected with *H.pylori* compared with uninfected patients or normal mucosa subjects($p<0.01$). The number of D-cells was significantly higher in uninfected patients and normal mucosa subjects patients compared with *H.pylori* infection ($p<0.01$). In patients with chronic gastritis the presence of CagA were associated with higher expression of G cell and not influence to D cell. **Conclusions:** the results of this study was strongly suggest that *H.pylori* infection induction protein expression of G-cell and reduction of protein expression of D-cells and the *H.pylori* positive CagA might play a role in up-regulation of the

[+] **Date received 7 / 9 / 2008 Date Accepted 29 / 4 / 2009**
[*] **Lecturer Health and medical technical College**

expression of G- cells but not effects on the expression of D-cells. The resulting relative hypofunction of the inhibitory action of D-cells against G-cells might be one of the reasons for the existing hypergastrinemia.

Introduction:

The Gram negative bacterium *Helicobacter pylori* (*H.pylori*) colonies, the gastric mucosa at the antrum in particular. *H.pylori* infection has several sequelae, including antral gastritis, chronic atrophic gastritis and duodenal ulcer, gastric adenocarcenoma and MALToma. [1, 2]. The wide spectrum of diseases from the same microorganism may depend on differences in the host response, but also on differences between the strains of *H.pylori* causing infection [3]. Two types of *H.pylori* have been described: type one produces a vacuolating cytotoxin which plays a fundamental role in pathogenicity [4]. This cytotoxin is not produced by type II strains. The cytotoxine associated with antigen (CagA) gene which also found in type I strains. Encoding a highly immunogenic protein, this gene is completely absent in type II strains. The CagA positive strain cause more server inflammations [5, 6, 7].

Helicobacter pylori infection interferes with the physiological control processes, resulting in alterations of gastric acid secretion. *H.pylori* infection results in a cascade of biochemical event in the gastric mucosa, including enhanced gastrin release after meal caused by reduced somatostatin production [8, 9]. The secretion of acid is an important function of the human stomach. In humans, although the rate of secretion varies, acid is continuously secreted by the gastric mucosa. During periods of fasting, the rate of acid secretion is low but sufficient to maintain an intragastric pH below 2 [10, 11]. The regulation of gastric acid secretion is achieved by the interplay between two major gastric endocrine cells: the gastrin G cell and the somatostatin D cell. Regulation of these cells occurs via stimulatory or inhibitory paracrine, endocrine, and neural pathways [12]. When food enters the stomach, the protein component stimulates G cells situated in the antral region of the stomach to release the hormone gastrin, which stimulates the enterochromaffin-like cells to release histamine and stimulates parietal cells to secrete acid. When the acidity of the stomach and duodenum increases, protective feedback pathways are activated to inhibit further acid secretion [13].

The relationship between *H.pylori* infection, gastric acidity and plasma gastrin levels, some evidence of an increase in gastrin and gastric acid secretion due to *H.pylori* infection (14). Gastrin, which is secreted by the G cells in the gastric mucosa then secreted into both the blood circulation and stomach lumen, enhances HCl secretion from the gastric parietal cells [15, 16]. There are different opinions as to how *H.pylori* infection contributes to gastroduodenal lesions. Goodwin and colleagues have suggested that *H.pylori* disrupts the local mucosal defense, which leads to gastroduodenal damage [17], while others noted that *H.pylori* infection increases gastric acid secretion, which in turn causes mucosal damage and the *H.pylori* infection blocks the normal inhibitory pathway through G cells [18]. The aim of this study were to determine whether there is an association between the *H.pylori* status, the numbers and function of gastric mucosal D, G in the gastric mucosa, to clarify the mechanisms of altered acid secretion as a result of infection.

Patients and Methods:

Subjects: The study included 70 gastritis patients and 50 apparently healthy controls with a mean age of 50.3 years (range between 20-75 year). The study was carried at the gastrointestinal unit at Al-Yarmook Teaching Hospital, and informed consent was obtained from all patients and controls . Two antral biopsies were obtained. The paraffin embedded blocks biopsies were used for histological evaluation and immunological staining for gastrin and somatostatin and another biopsy was used for the rapid urease test.

Histological evaluation:

The one biopsy specimen from the antrum of the stomach was fixed in 10% formalin then processed paraffin wax embedded section was cut into (3-4μm)thick section and stained with haematoxylin and Eosin. Giemsa staining was performed to detect or absence of *H.pylori* were assessed by the pathologist, who were unaware of the clinical and endoscopic finding.

Rapid urease test (RUT)

One biopsy was inoculated on urea agar slant, and then incubated at 37 C for (15 min-1hr) . Slant was examined for color change from yellow to pink if the bacteria was present secret urease and hydrolysis the urea to ammonia and raises the pH of the medium which change the color

All subjects were divided into three categories on the basis of histology and the results of the rapid urease test as those with: *H pylori* positive gastritis, *H pylori* negative gastritis, and histological normal gastric mucosa. Patients were classified as positive for *H pylori* if these two tests (RUT and Giemsa staining) were positive and as negative if both tests were negative.

In situ hybridization (ISH) for detection of *H.pylori* / CagA gene.

In situ hybridization (ISH) is a technique makes use of the high specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell. For detection of this markers , the biotinylated DNA probe hybridize to the target sequence (*H.pylori* / CagA mRNA sequence) then a streptavidin-AP (streptavidin-alkaline phosphatase) Conjugate is applied followed by addition of the substrate promo-chloro – indolyl – phosphatel / nitro-blue tetrazolium (BCIP/NBT) which yield an intense blue – black signal appears at the directly specific site of the hybridized probe. This strepteividin – Ap conjugate like the biotinylated probe provides raid and highly sensitive detection method [19;20].

The use of Biotin –Labeled DNA probe for *H.pylori* / CagA (8 μg/10015 μl ddH2O). Probe size: 349 bp (Maxim Biotech, Inc., U.S.A).

Hybridization /Detection System will give an intense blue –black color at the specific sites of the hybridization probe in both positive test tissues. Evaluation of the ISH signaling was done with the assistance of a histopathologist. The observer

was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers positive or negative cases, positive cells in the gastric tissue which gave a blue-black nuclear staining under the light microscope. The extent of the ISH signaling the cells of the examined tissue was determined in 10 fields under power microscope (100X) [21].

Immunohistochemical analysis (IHC) for detection of G and D cells

Mucosal biopsies were immunostaining with polyclonal antibodies to gastrin and somatostatin by the avidin-biotin complex (DakocCrop, Denmark). The primary antibody reacts with antigen in the tissue, and then a biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary antibody will form a complex with the peroxidase-conjugated streptavidin and by adding the substrate, which contains 3,3 ′-diaminobenzidine (DAB) in a chromogen solution, a brown-colored precipitate will form at the antigen site.

In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark red coloration of the cell nuclei [13].

The use of universal DakoCytomation streptavidin- biotin system purchased from DakoCytomation (USA) Immuno-histochemistry detection kit. The rabbit anti-human antibodies against gastrin and the rabbit anti-human antibodies against somatostatin were from DakoCrop (Denmark).

Counting the number of positive cells which gave brown cytoplasmic staining system under light microscope. The extent of the IHC signal was determined in 10 fields (X100magnification). In each field the total number of cells was counted and the extent of cytoplasmic staining cells was determined as a percent. The total staining score was divided by the number of whole cells per field in 10 fields, so the percentage of positively stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cell in the 10 fields [13].

Statistical analysis

Statistical analysis was performed using ANOVA test to determine whether the means were equal among three groups – i.e. CagA-, CagA+ and controls, p value of < 0.05 was considered statistically significant.

Results:

This work was performed on 120 subjects referred to the gastrointestinal endoscopy unit at Al-Yarmook Teaching Hospital. The patients profiles are listed in **(Table 1).**

The Rapid urease test and Giemsa staining showed that *H. pylori* infection was detected in 53 (75.71%) from 70 patients with both procedure, whereas the patients without *H.pylori* were 17 (24.28%) **(Table 2).**

In addition ,**Table 3** and **Figure 1** shows the expression of CagA was detected by in situ hybridization technique. From 53 patients complaining chronic gastritis and infected with *H.pylori* who were tested for CagA, 37 (69.81%) were found to be positive CagA and 16 (30.18%) patients have CagA – negative.

The results in **Table 4** and **5, Figure 2** and **3** compares the immunoreactive G cells and D cells in each group. The highly significant increased number of immunoreactive G cells was found among *H pylori* infected patients compared wit with uninfected patients or normal mucosa ($p<0.001$) . In contrast, the highly significant increased immunoreactive D cells was seen in normal gastric mucosa and uninfected patients compared with *H pylori* infected patients($p<0.001$). The mean percents of immunoreactive D cells in patients with *H pylori* positive chronic gastritis and with normal mucosa were not significantly different($p>0.05$). The number of immunoreactive G cells was higher and the number of D cells lower in those with *H pylori* infection compared with the *H pylori* negative group.

Furthermore, the *H pylori* infected individuals had significantly higher numbers of immunoreactive G cells in patients with *H.pylori* positive CagA than negative CagA and not influenced the numbers of immunoreactive D cells in patients with *H.pylori* positive CagA than negative CagA **(Table 6).**

Table (1): characteristics of 120 subjects used in the current study.

Variable	No.	(%)
Chronic gastritis	**70**	**58.33**
Healthy subjects	**50**	**41.66**
Total	**120**	**100**

***H.pylori* status**	**No.**	**(%)**
***H.pylori* - positive**	**53**	**75.71**

***H.pylori* - negative**	**17**	**24.28**
Total	**70**	**100**

Table (2): The prevalence of *H.pylori* infection in patients with chronic gastritis depend on Rapid urease test and Giemsa staining.

Table (3): Expression of CagA mRNA in *H.pylori* –positive patient with chronic gastritis by (ISH).

Group	**CagA status**	**No.**	**(%)**
***H.pylori* -positive**	**positive**	**37**	**69.81**
	negative	**16**	**30.18**
	Total	**53**	**100**

Groups	**No.**	**Mean(%) ± SE**	***p* value between groups**
***H.pylori*(+ve)**	**53**	**75.2 ± 3.2**	**Control — *H.pylori*(+ve)****
***H.pylori* (-ve)**	**17**	**51.4 ± 2.5**	**Control — *H.pylori* (-ve)****
Control	**50**	**22.8 ± 0.6**	***H.pylori* (+ve) — *H.pylori* (-ve)****

Table (4): Expression of G cell in patients with chronic gastritis(*H.pylori* positive and negative) and healthy volunteers.

**= highly significant difference(p<0.01); (+ve)=positive ; (-ve)=negative

Table 5: Expression of D cell in patients with chronic gastritis(*H.pylori* positive and negative) and healthy volunteers.

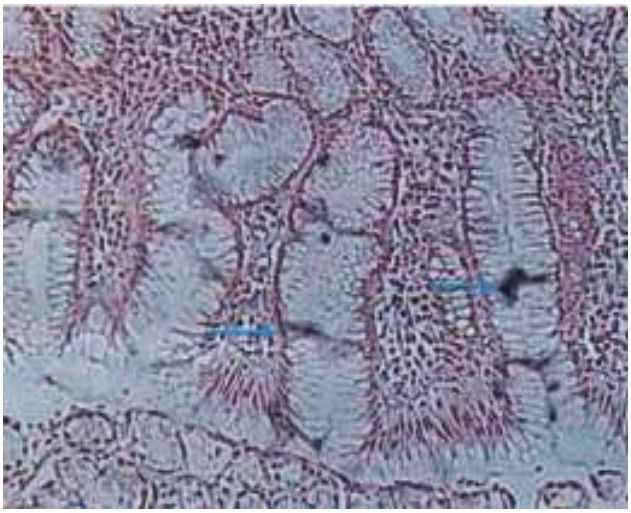
Groups	**No.**	**Mean(%) ± SE**	***p* value between groups**
***H.pylori*(+ve)**	**53**	**23.2 ± 5.2**	**Control — *H.pylori*(+ve)[n]**
***H.pylori* (-ve)**	**17**	**38.5 ± 4.5**	**Control — *H.pylori* (-ve)****
Control	**50**	**20.1 ±3.7**	***H.pylori* (+ve) — *H.pylori* (-ve)****

[n]= not significant(p>0.05) ;**= highly significant difference(p<0.01); (+ve)=positive ; (-ve)=negative

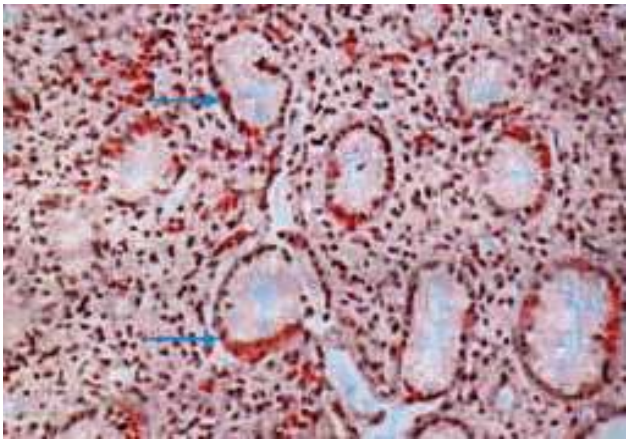
Table 6: Expression of G cell in patients with chronic gastritis and healthy volunteers.

Variables	*H.pylori* (+ve) CagA status	No.	Mean(%) ± SE	*p* value
G cell	CagA +	37	72.4±5.1	$p<0.01$
	CagA -	16	54.3± 3.8	
D cell	CagA +	37	30. 5±1.9	$p>0.05$
	CagA -	16	35.2 ± 2.2	

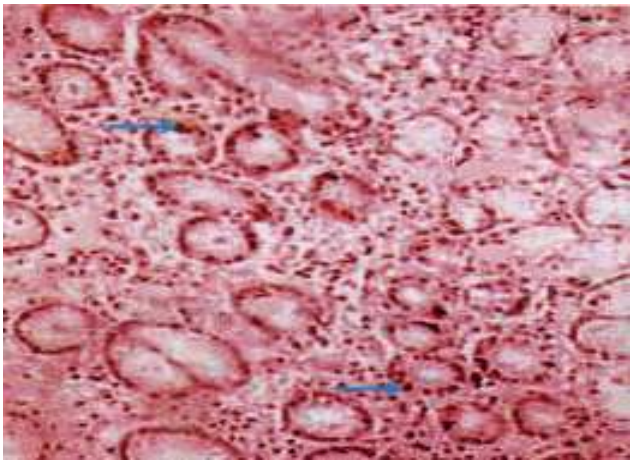
($p<0.01$)= highly significant difference; ($p>0.05$) = not significant



Figure(1):Detection of CagA in patients with chronic gastritis disease by in situ hybridization(ISH). Staining of CagA mRNA by BCIP/NBT(blue-black),counterstained with nuclear fast red. Tissue from patients with antral gastritis shows positive CagA by hybridization signal.



Figure(2):Immunohistochemical staining(IHC) by DAB chromogene (dark brown), counterstained with nuclear fast red. Antral mucosa stained for gastrin positive G cells.



Figure(3):Immunohistochemical staining(IHC) by DAB chromogene (dark brown), counterstained with nuclear fast red. Antral mucosa stained for somatostatin positive D cells.

Discussion

Helicobacter pylori associated with chronic mucosal inflammation might alter normal gastric physiology, and peptic acid secretion, either directly or through alterations to cells involved in the regulation of the gastric secretary process, such as antral G or D cells, enterochromaffin-like (ECL) cells [22]. Gastrin G and somatostatin D cells are the major endocrine cells in the stomach known to play an important role in acid secretion [8].

The G and D cells counts in the antrum were assessed in this study. A higher G cell count and lower D cells were established in patients with chronic gastritis in course of *H.pylori* infection in comparison with patients without infection and healthy subjects. These results are comparable to other data were rapidly confirmed, and it was also shown that this increase was reversed after *H pylori* eradication [23, 24, 25]. That confirmed the cell counts of G and D cells were altered in *H pylori* infected gastric mucosa, with an increase in the proportion of G cells and a decrease in D cells. But other reports assessing G cell counts in chronic gastritis patients that did not discover significant differences in G cell count between patients with or without infection. Although D cell count was lower in the infection [26; 27].

Moreover, in the present study was found that 74% of patients who have *H.pylori* infection have CagA positive strains. This results in agree with previous study that showed the *H.pylori* strains were positive for the CagA in 74.4% of Costa Rica patients [28]. Other study reported the prevalence of CagA was more than 80% among patients with chronic gastritis [6]. Increasing of CagA mRNA among those patients may explain the role of CagA positive *H.pylori* in the development of gastritis [29]. The mechanisms by which CagA modify the activity of epithelial cells is explaining by serving as scaffolding protein able to interact and modify the function of a variety of molecules involved in cell to cell interaction, cell motility, and proliferation [6;30].

The CagA positive *H.pylori* strains played a major role in causing the infection and a higher levels of G cell count were associated with CagA, while somatostatin was not influenced by CagA positive *H.pylori* strains. Possibly through gastrin stimulation *H.pylori* infection sustained by CagA strains may cause sever gastric damage than strains without CagA, since the later may not only cause less extensive epithelial damage, but may also favors the defense mechanisms of the stomach against mucosal injury [31; 32]. The hypergastrinemia in *H.pylori* associated gastritis is relevant to the presence of CagA, and the possible mechanism of hypergasrinemia may be related to antral D-cell deficiency, which caused by *H.pylori* infection with expression of CagA [32].

The fact that there was an association with the severity of inflammation is more consistent with a role for cytokines released by inflammatory cells (for example, interleukin -1b, tumor necrosis factor and interferon- γ). The increase in inflammation associated with presence of more virulent *H.pylori* type containing CagA would have a more profound effect on gastrin / somatostatin homeostasis [33].

In conclusions**:** the results of this study was strongly suggest that *H.pylori* infection induction protein expression of G-cell and reduction of protein expression of D-cells and the *H.pylori* positive CagA might play a role in up-regulation of the expression of G- cells but not effects on the expression of D-cells.The resulting relative hypofunction of the inhibitory action of D-cells against G-cells might be one of the reasons for the existing hypergastrinemia.

References:

1- Pinto-Santini, D. and Salama, N.“ The biology of H.pylori infection, a major risk factor for gastric adenocarcinoma”. *Cancer Epidimiol. Biom. Prev*., 14: 1853- 858, (2005).

2-Versalovic, J. “ H.pylori pathology and diagnostic strategies”. *Am. J. Clin. Pathol*., 119:43-412,(2003)

3- Blaser, M. J. and Atherton, J. C. “ H.pylori persistence: biology and disease”. *J. Clin. Invest*., 113: 321-333,(2004)

4- Lin, C. Chang, Y.; Wu, S. and Chen, D.; Lin, s.; Lin, J.; Sun, Y. and Chow, P. “Comparative immunoproteomics of identification and characterization of virulence factors from *H.pylori* related to gastric cancer”. *Mol. Cell. Proteom*.; 5: 1484-1496. (2006).

5-Bebb, J.; Letley, D.; Thomas, J.; Collins, H.; Hand, N. and Atherton, J. “*H.pylori* up regulates matrilysin (MMP-7) in epithelial cell in vivo and in vitro in Cag dependent manner”. *Gut*; 52: 1408-1413, (2003).

6- Backert, S.; Schwartz, T.; Kirsch, C.; Sommer, C.; Kwok, T.; Gerhard, N.; Goebel, U. and Koenig, W. “Functional analysis of the Cag pathogenicity island in H.pylori isolates from patients with gastritis, peptic ulcer and gastric cancer”. *Infect. Immun.*, 72: 1043-1056, (2004).

7- Smith, G.; Hold, G.; Tahara, E. and El-Omer, E. “Cellular and molecular aspects of gastric cancer”. *World J. Gastroenterol*.; 21: 2979- 2990,(2006).

8- Huang, X. “Helicobacter pylori infection and gastrointestinal hormones: a review”. *World J. Gastroentero*.; 6:783-788. (2000).
9- Ching, C. “Helicobacter pylori infection: Basic and clinical problems”. *J. Gastroenterology and Hepatology*. 16:276-281, (2001).
10- Honarkar, Z.; Baladast, M.; Ehsani, J.; Jodeiri, B.; Seyedi, Sh and Zali, R. “Gastrin, Cholecystokinin (CCK) and H.pylori in nonulcer Dyspepsia”. *Shiraz E-Medical Journal.* 8: 538-541, (2007).
11-Buljevac, M.; Banic, M.; Kujundzic, M.; Dominis, M.; Colic, V.; Kardum, D and Katicic, M. “Non-ulcer dyspepsia and Helicobacter pylori infection”. *Lijec Vjesn*.; 124 Suppl 1:61-3, (2002).
12- Gurbuz, K.; Kucukkardali, Y.; Yazgan, Y.; Özel, M. and Polat, T. “Does eradication of Helicobacter pylori infection reduce hypergastrinema during long term therapy with proton pump inhibitors? ” *The Turkish Journal of Gastroenterology*. 13:159-163,(2002).
13- Liu, Y.; Vosmaer, C.; Tytgat, G.; Xiao, S. and Kate J. “ Gastrin (G) cells and somatostatin (D) cells in patients with dyspeptic symptoms: Helicobacter pylori associated and non-associated gastritis”. *Journal of Clinical Pathology*. 58:927-931. (2005).
14- Kamada, T.; Haruma, K.; Hata, J.; Kusunoki, H.; Sasaki, A.; Ito, M.; Tanaka, S. and Yoshihara, M. “The association between antral G and D cells and mucosal inflammation, atrophy, and Helicobacter pylori infection in subjects with normal mucosa, chronic gastritis, and duodenal ulcer”. *Am J Gastroenterol.* 93: 748-752,(1998).

15- Peterson, L.; Barnett, C. and Evans, D. “Acid secretion and serum gastrin in normal subjects and patients with duodenal ulcer: the role of *Helicobacter pylori*”. *Am J Gastroenterol*.; 88:2038–2043. (1993).
16- Gibbons, H.; Legon, S. and Walker, M. “The effect of gastrin-releasing peptide on gastrin and somatostatin messenger RNAs in humans infected with *Helicobacter pylori*”. *Gastroenterology*. 112:1940–1947, (1997).
17- Goodwn, C. “Duodenal Ulcer, Campylobacter pylori and the "Leaking-Roof" Concept”. *Lancet*. 2: 1467,(1988).
18- Sumii, M.; Summi, K. and Tari, A. “Expression of antral gastrin and somatostatin mRNA in *Helicobacter pylori*-infected subjects”. Am J Gastroenterol.; 89:1515–1519, (1994).
19- Maritette, P.C.; Roeland. H.D.; Rob, P. M.,G.; Van Erica, B.; Clavida, M.H.; Roelof, A.P.; Jim, E.L. and Anton, K.R. “Sensitive mRNA detection fluorescence in situ hybridization using horseradish peroxidase – labeled Oligodeoxy nucleotides and tyramid signal amplification”. *J. Histochem. Cytochem*.; 46: 1249-1259, (1998).
20-Yoshiyyki, R.O.; Yoshiko, I. and Akira, M. “Aplication of plastic embedding to electron microscopic immunocytochemistry and in situ hybridization in observation of production and secretion of peptide hormones”. *J. Histochem. Cytochem*.; 98: 885-891, (2002).
21-Camorlinga – ponce, M; Romo, C.; Gonzalez – Valencia, G.; Munoz, O. and Torres, J. “Topogaraphical localization of CagA positive and CagA negative Helicobacter pylori strains in the gastric mucosa; an in situ hybridization study”. *J. Clin. Pathol*.; 57:822-828, (2004).
22- Zavros , Y.; Rieder , G.; Ferguson , A.; Samuelson , L and Merchan , J. “Hypergastrinemia in response to gastric inflammation suppresses somatostatin”. *Am J Physiol astrointest Liver Physiol*.; 282: 175-183, (2002).

23- Furata, T.; Baba, S. and Takashima, M. " Effects of Helicobacter pylori infection on gastric juice pH". *Scand J Gastroenterol* .; 33:357–363, (1998).
24- Gisbert, J.;Boixeda, D. and Al-Mostafa, A. "Basal and stimulated gastrin and pepsinogen levels after eradication of Helicobacter pylori: a 1-year follow-up study". *Eur J Gastroenterol Hepatol* .;11:189–200, (1999).
25- Iijiman, K. Ohara, S. and Sekine, H. "Changes in gastric acid secretion assayed by endoscopic gastric test before and after Helicobacter pylori eradication". *Gut.* 46:20–26, (2000).
26- Tzaneva, M. "Light and electron microscopic immunohistochemical investigation on G and D cells in antral mucosa in Helicobacter pylori-related gastritis". *Exp Toxicol Pathol*.; 52:523–528, (2001).
27- Park, S.; Lee, H.; Kim, J.; Park, J.; Jung, G.; Han, S.; Cho, J. and Kim. "Effect of *Helicobacter pylori* infection on antral gastrin and somatostatin cells and on serum gastrin concentrations". *Korean J Intern Med.* 14: 15-20, (2001).
28- Occhialini, A.; Marais, S.; Urdaci, M.; Serra, R.; Munoz, N.; Covacci, A. and Megraud, F. "Composition and gene expression of the CagA pathogenicity isolated from gastric carcinoma and gastritis patients in Costa Rica". *Infect. Immun*.; 94:1902-1908. (2001).
29- Nomura, Y.; Perez-Perez, G.; Lee, J.; Stemmermann, G. and Blaser, J. " Relation between *H.pylori* CagA status and risk of peptic ulcer disease". *Am. J. Epidemiol*.; 55: 1054-1059, (2002).

30- Moese, S.; Selbach, M. and Kwok, T. "*H.pylori* induces AGS cell motility and elongation via independent signaling pathways". *Infect. Immun*.; 72: 3646-364, (2004).
31- Basso, D.; Navaglia, F.; Brigato, L.; Mario, F.; Rugge, M. and Plebani, M. "*Helicobacter pylori* non-cytotoxic genotype enhances mucosal gastrin and mast cell trypase". *J. Clin. Pathol*.; 52: 210-214, (1999).

32-Kim, J.; Park, H.; Cho, J. Lee, K.; Lee, S.; Park, I. and Kim, C. "Relationship of CagA to serum gastrin concentration and antral G, D cell densities in *H.pylori* infection. " *Yonsei Med. J*.; 40: 301-306,(1999).

33- Blaser, M. "Hypotheses on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation". *Gastroenterology*. 102:720–727, (1992).