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ORIGINAL ARTICLE

Urocortin and Cytokines in Gastric Mucosa: Relationship to Inflammatory Activity before and after Medical Eradication of *Helicobacter Pylori*

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ABSTRACT

Background: There is a debate whether the majority of gastritis mucosal injury occurs as a direct effect of *Helicobacter pylori (H. pylori)* infection or as a result of immune cell damage from abundant mediators of inflammatory response. Recently, Urocortin (UCN) has emerged as a mediator of gastrointestinal (GI) responses to noxious stimuli. Interleukins-1β (IL-1β) and interleukin-10 (IL-10) may play important roles in gastric inflammation caused by *H. pylori* infection.

Aim of the Work: We aimed to localize UCN 1, IL-1β, and IL-10 in gastric biopsies to elucidate their possible role in the local inflammatory response before and after medical eradication of *H. pylori* infection.

Patients and Methods: Gastric biopsies were obtained from 72 patients with dyspeptic symptoms. Specimens were examined for gastritis, presence or absence of *H. pylori* and immunohistochemically for UCN-1, IL-1β and IL-10 expression.

Results: *H. pylori* was positive in 39/72 (54.2%) cases. There were significant differences in antral activity and inflammatory scores, UCN-1 and IL-1β expression between *H. pylori* positive and *H. pylori* negative patients ($P<0.002$, <0.03, <0.002 and <0.03 respectively). In *H. pylori* positive patients; eradication therapy has significantly reduced activity and chronicity of gastritis, increased UCN-1 and reduced IL-1β and IL-10 immunostaining ($P<0.002$, <0.002, <0.001, <0.000 and <0.000 respectively). There were significant negative, positive and no correlation between activity of gastritis and UCN-1, IL-1β ($P<0.03$ and <0.02) and IL-10 immunostaining respectively.

Conclusion: UCN-1 may be involved in the local anti-inflammatory process in active gastritis. IL-1β is considered a marker of inflammation as it increased with increasing severity of gastritis and markedly decreased after treatment.

Key Words: Gastritis, *H. pylori*, gastritis, Urocortin, interleukin 1β and interleukin -10.

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ABBREVIATIONS

Helicobacter pylori (H. pylori), Urocortin (UCN), gastrointestinal (GI), interleukin 1β (IL-1β), interleukin-10 (IL-10), interleukins (ILs), polymorph nuclear leucocytes (PMC), mononuclear cells (MNC), tumor necrosis factor alpha (TNF-α), corticotropin releasing hormone

(CRH), gastrointestinal tract (GIT), corticotropin releasing factor (CRF), Campylobacter-like organism test (CLO test), Hematoxylin and Eosin (H&E), Catalogue number; Cat #, normal goat serum (NGS), minutes (min), phosphate buffered saline (PBS), diaminobenzidine (DAB), T helper (Th).

INTRODUCTION

Gastritis has long been recognized as a common finding in normal population and its histological presence is closely paralleled by the presence of *H. pylori*. The frequency of *H. pylori* gastritis is related to the age and the geographic location *(SIurala et al. 1968)*.

H. pylori is predominantly an extracellular organism usually lives in/or beneath the gastric mucous layer adjacent to the epithelial surface and close to the intercellular junction *(Andersen et al. 1987 & Marshall et al. 1987)*. The antrum is its primary area of residence perhaps because it has the thickest mucous coat *(Rauws and Tytgat, 1990)*.

The key pathophysiological event in *H. pylori* infection is triggering of an inflammatory response and its main cytokine mediators interleukins (ILs). *H. pylori* associated gastritis is characterized by marked infiltration of the gastric mucosa by polymorph nuclear leucocytes (PMC) and mononuclear cells (MNC) *(Rauws et al. 1988 and Testerman et al. 2001)*. Accumulation and activation of these cells were suggested to be induced by local production of cytokines. Mucosal levels of ILs as IL-1 β, IL-6, IL-8, IL-10 and tumor necrosis factor alpha (TNF-α) were significantly higher in *H. pylori* positive than in *H. pylori* negative patients *(Noach et al. 1994 and Yamaoka et al. 1996 & 1997)*. Taken together, these results suggest that these cytokines may play an important role in gastric inflammation caused by *H. pylori*.

The corticotropin releasing hormone (CRH) and its related family of neuropeptides; UCN and their corresponding receptors are detectable at multiple sites along the whole length of the gastrointestinal tract (GIT). Their presence, mainly UNC-1, regulation of expression and the relation to corticotropin releasing factor (CRF) receptors have been extensively characterized in the heart, skeletal muscle, skin and immune system, while less is known in the gut *(Chen et al. 2004 and Slominski et al. 2004)*. Many reports suggest that CRH peptides play important physiological roles in the regulation of GI motility and GI response to noxious stimuli. However, it now appears that the distribution of members of the CRH family of neuropeptides and their receptors along the GIT differs, resulting in distinctive physiological effects. Thus, activation of the predominant CRH-R1 receptors in the colon results in stimulation of its propulsive activity, whereas activation of the predominant CRH-R2 receptors in the stomach leads to inhibition of the gastric emptying rate *(Martinez et al. 2002)*. The fact that both Urocortin ligands and CRF receptors are expressed in the GIT support the local action of UCNs in modulation of GI functions *(Kozicz and Arimura, 2002 & Martinez et al. 2004)*.

The presence of UNC-1, 2 and 3 mRNA in the GIT was first reported by *in situ* hybridization and RT-PCR *(Bittencourt and Sawenko, 2000)*. Urocortin 1 immunoreactivity was initially identified in rat duodenal extracts *(Vaughan et al. 1995)* and later in human gastric tissue *(Chatzaki et al. 2003)*. Immunohistochemical studies showed that the peptide is present in the gastric mucosa (in parietal and other unidentified cell types) *(Chatzaki et al. 2003 and Martinez et al. 2004)*.

AIM OF THE WORK

Thus, our aims were to localize the UNC-1, IL-1β and IL-10 in gastric biopsies to elucidate their

possible involvement in the local inflammatory response before and after medical eradication of *H. pylori* infection.

PATIENTS AND METHODS

This study included 72 patients; 32 men and 40 women with age range 20-65 years and mean age of 40.1 years who were referred to Endoscopy Unit, Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Sohag University. Patients were admitted for dyspeptic symptoms including abdominal pain, heart burn, fullness, nausea, vomiting, regurgitation and anorexia. Special habits reported were: Tea consumption in 63 (87.5%), smoking in 24 (33.3%) and eating spicy food in 51 (70.8%) patients. None of the patients had received non-steroidal anti-inflammatory drugs, proton pump inhibitors, or antibiotics within the previous three months prior to the study. The other exclusion criteria include; history of duodenal or gastric ulcer, GERD, gall bladder or bile duct lithiasis, pancreatitis, cirrhosis, inflammatory bowel disease and cancer. All patients gave an informed consent before inclusion in the study and the study was approved by the Local Ethics Committee.

All endoscopies were reported according to the criteria of endoscopic gastritis described by the Sydney classification, which include assessment of severity as mild, moderate or severe according to *Tytgat et al. (1991)*. Then endoscopic findings were classified by modifying the following eight categories of: Erythematous/exudative gastritis, atrophic gastritis, raised erosive gastritis, flat erosive gastritis, hemorrhagic gastritis, rugal hyperplastic gastritis, enterogastric reflux gastritis and congestive gastroenteropathy according to *Khakoo et al. (1994)*. For simplicity our modification divides gastritis into three categories; erythematous, erosive and atrophic.

An initial gastroscopy was performed for all patients and four gastric mucosal biopsies were obtained from the corpus of the stomach and pyloric antrum. One antral biopsy was immediately tested for *H. pylori* by Campylobacter-like organism test; CLO test (Kimberly Clark test; Rapid Urease Test, 25/cs, Item #: KC-60480, Roswell, GA, USA). The test was performed at the time of gastroscopy. A biopsy of mucosa was taken from the antrum of the stomach and was placed into a medium containing urea and an indicator such as phenol red. The urease produced by *H. pylori* hydrolyzes urea to ammonia, which raises the pH of the medium and changes the color of the specimen from yellow (NEGATIVE) to red (POSITIVE).

In *H. pylori* positive patients, a follow up gastroscopy was performed two months after eradication treatment, consisting of (amoxaicillin; 1gm, clarithromycin; 500 mg, omerprazole; 20 mg, orally twice daily for 10 days each and then omeprazole; 20 mg daily for 1 month).

Histopathology: Gastroscopic formalin fixed biopsies were sent to the Pathology Laboratory of Sohag University Hospital to be processed. Five-micron tissue sections were cut from the paraffin embedded tissues, stained with Hematoxylin and Eosin (H&E) and examined by a pathologist using bright field microscope. Patients were classified into two groups according to the absence or presence of endoscopic, histopathological findings (presence or absence of gastritis, activity and/or chronocity). *H. pylori* status was determined by the presence or absence of *H. pylori* organism in the mucous inside the glands in H&E stained sections, in addition to CLO urease test into *H. pylori* gastritis (39 cases) and non- *H. pylori* gastritis (33 cases). Patients were classified as *H. pylori* positive if at least one of those methods gave positive result.

Grading of gastritis was scored as modified from the Sydney System classification *(Dixon et al. 1996).* Activity score was measured by counting PMC infiltration and the inflammatory

score was measured by MNC infiltration. Recorded separately; a grade from 0-3 for none, mild, moderate and marked infiltration, respectively was done to determine the activity and/or chronicity; inflammatory score of gastritis.

Immunohistochemistry: Serial 5 μm tissue sections on pre-cleaned (Superfrost®*/Plus-Fisherbrand®-USA) glass slides were stained using Sreptavidin-Biotin-Peroxidase complex method as described by the manufacturer. The primary antibodies used were UNC-1 rabbit polyclonal antibody (Catalogue number; Cat# RB-9415-P0; 0.1ml, LabVision Corporation, Westinghouse, USA), IL-1β mouse monoclonal antibody (Cat# AF3747; 0.1ml, R&D Systems Inc, Minneapolis, USA) and IL-10 rat monoclonal antibody (Cat # RT-9461-P0; 0.1ml, LabVision Corporation, Westinghouse, USA). The Envision Kit used was Universal staining kit (Cat # TA-015-HP, LabVision Corporation, Westinghouse, USA).

The sections were deparaffinized in xylene and rehydrated in descending concentrations of ethanol. Endogenous peroxidase activity was blocked by treating sections with 0.3% H_2O_2 in methanol. Nonspecific antibody binding sites were blocked by incubating tissue sections with normal goat serum (NGS) for 30 minutes (min). Tissue sections were washed in phosphate buffered saline (PBS) pH 7.4. Antigen retrieval was achieved by boiling tissue sections for 5 min in 0.01 M citrate buffer pH 6.0, in a Coplin Jar in microwave oven at 600W, allowed to cool to room temperature, equilibrated in PBS for 10 minutes.

Primary antibodies were applied at dilutions of 1:50, 1:100 and 1:50 for UNC-1, IL-β and IL-10 respectively in a humid chamber at 4°C overnight. The resulting immune-complex was detected by applying the secondary antibody for one hour. After washing in PBS, sections were incubated in 14-diaminobenzidine (DAB) and 0.1% H_2O_2 for 10 minutes, washed in distilled water and counter-stained using Mayers Hematoxylin.

Positive controls consisted of sections from gastric carcinoma and colon carcinoma previously known to be positive for Urocortin, both IL-1β and IL-10, respectively. Negative controls included the use of non-immune NGS as the primary antibody.

Interpretation of the staining was made semi-quantitatively by assessing the mean percentage of the stained mucosal cells and the intensity of the stain in five microscopic fields at 400X magnification and classified into four grades; negative, mild, moderate, and strong immunoreactivity (0, +1, +2 +3, respectively).

Statistical Methods:

Statistical Package of Social Science SPSS; Version 10, Chicago, USA) was used for statistical analysis. Quantitative and semiquantitative variables as the histological activity and the inflammatory scores were presented as mean ± SD. Student's t- test was used for comparison between groups. Correlation between different variables was studied using correlation coefficient (r). P value less than 0.05 was considered statistically significant.

RESULTS

The endoscopy findings were classified into three categories; mild, moderate and severe and/or erythematous, erosive and atrophic (Table 1, Figure 1 A-C).

Rapid urease test and examination of H&E stained sections of gastric biopsies revealed the presence of *H. pylori* in 39/72 (54.2%) of cases whereas 33/72 (45.8%) were *H. pylori* negative. *H. pylori* bacteria were demonstrated in the mucous, on the cell surface and within gastric pits as rod shaped, pink spiral bacilli (Figure 1 D).

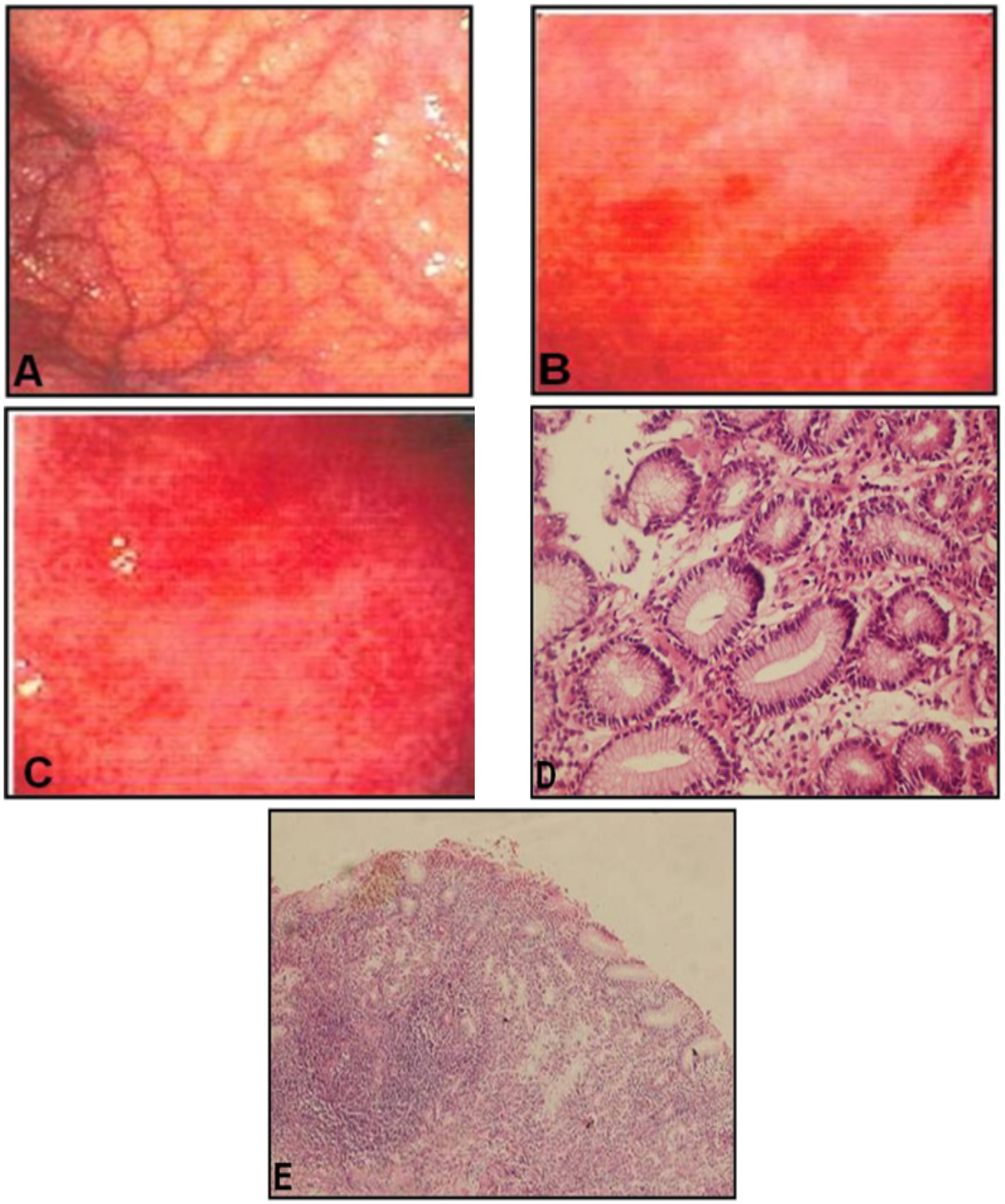

Figure 1: Endoscopic picture of atrophic (***A***), erosive (***B***) and erythematous gastritis (***C***) and H&E stained sections of *H. pylori* positive gastritis (***D***, X200) and chronic active gastritis (***E***, X100).

Table 1: Endoscopic findings of all cases of gastritis: according to *Tytgat et al. (1991)* and *Khakoo et al. (1994)*.

Grade of gastritis	Percentage %	Type of gastritis	Percentage %
1- Mild	51 (70.8%)	1- Erythematous	39 (54.2%)
2- Moderate	15 (20.8%)	2- Erosive	12 (16.7%)
3- Severe	6 (8.4%)	3- Atrophic	21 (29.2%)
Total	**72 (100%)**	**Total**	**72 (100%)**

Both PMC and MNC were detected in the lamina propria and attaching the glandular epithelium (Figure 1 E).

UNC-1, IL-1β and IL-10 were immunohistochemically expressed as yellowish brown stain in the cytoplasm of both surface epithelial cells and glandular epithelial cells. IL-1β and IL-10 were also expressed in the MNC infiltrate (Figures 2 A-F).

Activity score of gastritis was histopathologically graded according to PMC infiltration into mild, moderate and marked activity in 18 (46.2%), 12 (30.8%) and 9 (23%) cases, respectively. Chronicity of gastritis was histopathologically graded according to MNC infiltration into mild, moderate and marked in 15 (38.5%), 15 (38.46%) and 9 (23%) of cases, respectively (Table 2).

There were significant differences in the activity score between *H. pylori* positive and *H. pylori* negative cases in both antrum and corpus biopsies. Similarly, a significant difference in the inflammatory score was found between *H. pylori* positive and *H. pylori* negative cases in the antral biopsies only (Table 3). *H. pylori* eradication therapy has significantly reduced both antral and corporal activity and inflammation as determined by PMC and MNC infiltration (Table 4).

There was significant decrease in UNC-1 and increase in IL-1β immunostaining expression in *H. pylori* positive than in *H. pylori* negative cases, while there was no significant difference in IL-10 expression between *H. pylori* positive and *H. pylori* negative cases (Table 4). *H. pylori* eradication therapy has significantly increased UCN and significantly reduced IL-1β and IL-10 immunostaining expression in *H. pylori* positive patients (Table 5, Figure 3-5).

There was significant negative correlation between UCN and activity of gastritis. On the other hand there was significant positive correlation between IL-1β immunostaining and activity of gastritis. While no correlation was noticed between IL-10 immunostaining and activity score (Table 6). There were significant negative correlation between UCN immunostaining and inflammatory score and positive correlations between IL-1β, IL-10 immunoreactivity and inflammatory score (Table 7).

The interesting finding in this study was the presence of 9 specimens expressing strong IL-1β and IL-10 immunostaining. On the other hand, the degree of staining of IL-10 was higher in *H. pylori* positive specimens with low level of IL-1β than in *H. pylori* positive specimen over all. There were no correlations between UCN and either IL-1β or IL-10.

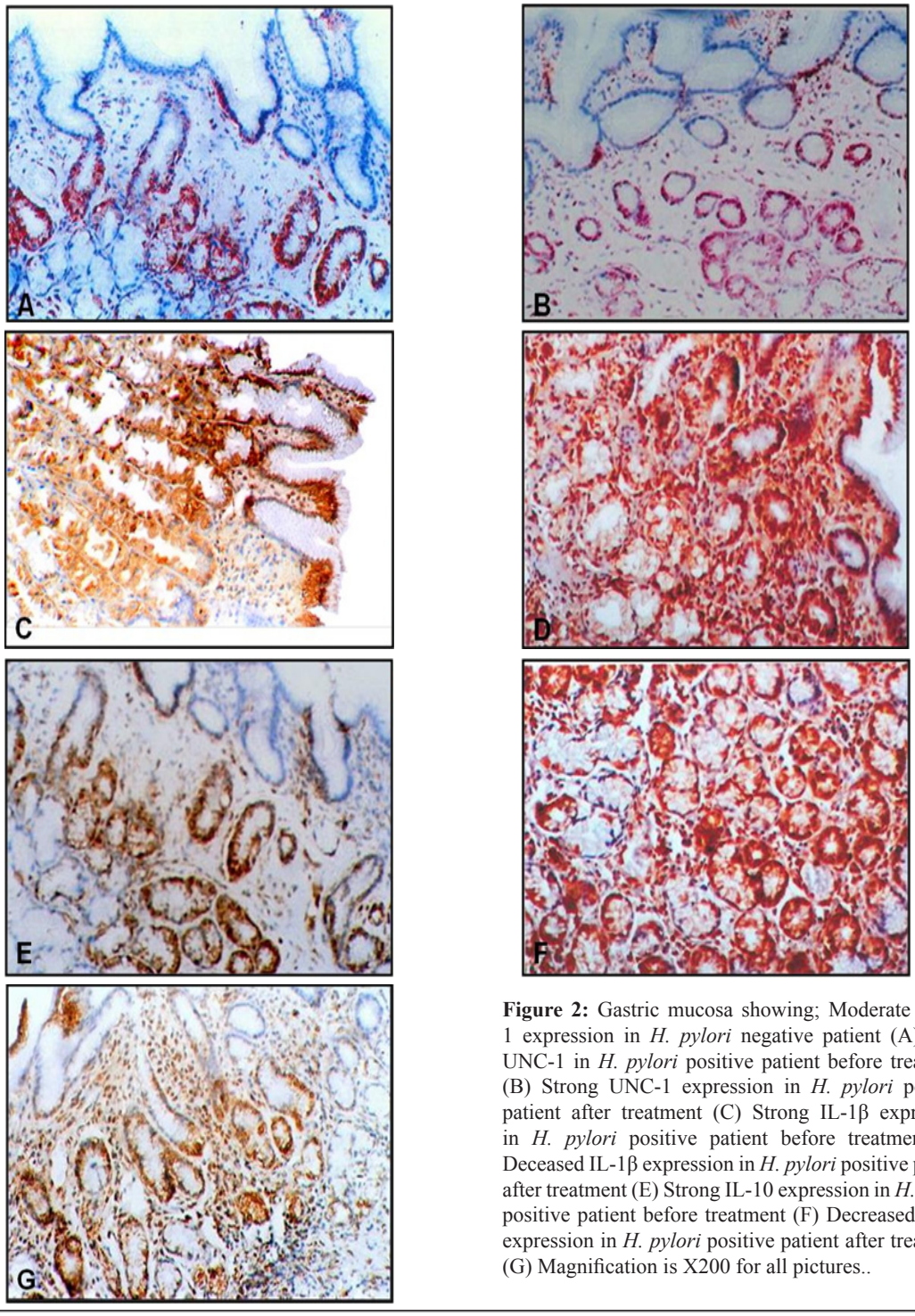

Figure 2: Gastric mucosa showing; Moderate UNC-1 expression in *H. pylori* negative patient (A) Mild UNC-1 in *H. pylori* positive patient before treatment (B) Strong UNC-1 expression in *H. pylori* positive patient after treatment (C) Strong IL-1β expression in *H. pylori* positive patient before treatment (D) Deceased IL-1β expression in *H. pylori* positive patient after treatment (E) Strong IL-10 expression in *H. pylori* positive patient before treatment (F) Decreased IL-10 expression in *H. pylori* positive patient after treatment (G) Magnification is X200 for all pictures..

Table 2: Activity and inflammatory scores in *H. pylori* positive and negative patients.

PMC and MNC infiltration	Positive cases	Negative cases	P value
• Activity score (corpus)	1.00±0.91	0.20±0.30	P <0.02
• Activity score (antrum)	1.00±0.08	0.02±0.30	P <0.002
• Inflammatory score (corpus)	2.10±0.89	1.9±1.42	NS
• Inflammatory score (antrum)	2.14±0.91	1.21±1.19	P <0.03

Table 3: Activity and inflammatory scores before and after eradication of *H. pylori*.

PMC and MNC infiltration	Before eradication	After eradication	P value
Activity score (corpus)	1.00±0.91	0.00±0.00	P <0.002
Activity score (antrum)	1.00±0.08	0.00±0.00	P <0.001
Inflammatory score (corpus)	2.10±0.89	1.05±0.87	P <0.002
Inflammatory score (antrum)	2.14±0.91	0.95±1.24	P <0.004

Table 4: UCN 1, IL-1β and IL-10 expression in *H. pylori* positive and negative patients.

Immunostaining expression	*H. pylori* (+)	*H. pylori* (-)	P value
UCN 1	1.08±0.28	1.27±0.19	P <0.002
IL-1β	2.62±0.51	1.28±1.08	P <0.03
IL-10	2.62±0.51	2.28±1.08	NS

Table 5: UCN 1, IL-1β and IL-10 expression before and after eradication of *H. pylori*.

Immunostaining expression	Before eradication	After eradication	Significance
UCN 1	1.08±0.28	2.38±1.12	P <0.001
IL-1β	2.62±0.51	0.46±0.52	P <0.000
IL-10	2.62±0.51	0.46±0.52	P <0.000

Table 6: Correlation of UCN 1, IL-1β and IL-10 with the activity score (PMC infiltration).

Immunostaining expression	Correlation coefficient (r)	Significance
UCN 1	-0.7	P <0.03
IL-1β	0.7	P <0.02
IL-10	0.25	NS

Table 7: Correlation of UCN 1, IL-1β and IL-10 with MNC infiltration (chronicity).

Immunostaining expression	Correlation coefficient (r)	Significance
UCN	-0.3	P <0.05
IL-1β	0.6	P <0.03
IL-10	0.7	P <0.02

NS: not significant.

DISCUSSION

H. pylori organism is the major recognized etiological agent inducing gastric inflammatory responses *(Dixon, 1991, Blaser, 1992 & Noach et al. 1994)*. These responses can be considered to have two components: An acute inflammatory response characterized by intraepithelial and interstitial PMC infiltration and chronic inflammatory responses associated with increased numbers of MNC in the lamina propria, including lymphocytes, monocytes/macrophages, and plasma cells *(Blaser, 1992)*. These two responses may be regulated differentially following induction of cytokines involved in the inflammatory cascade, including TNF-α, IL-1, IL-6 and IL-8 *(Crabtree et al. 1993, Gionchetti et al. 1994 and Ando et al. 1996)*.

In agreement with *Lopes et al. (2005)* the antral and corpal activity scores were significantly higher in *H. pylori* positive cases than in *H. pylori* negative cases in the current study. The antral inflammatory scores were higher in *H. pylori* positive than in *H. pylori* negative cases. While the corpal inflammatory score was higher in *H. pylori* positive cases than in *H. pylori* negative but the difference is not significant.

With *H. pylori* eradication, there is a substantial decrease in grades of activity and chronic inflammation as measured by quantitative analysis of the actual number of PMC and MNC present in the lamina propria in this study. The observations that PMC infiltration had completely disappeared and decrease of MNC infiltration after the end of successful therapy confirm the earlier studies in Western populations that histological resolution of PMC and MNC infiltration occurs differentially after *H. pylori* eradication *(Morgan et al. 1988 and Valle et al. 1991)*.

An active involvement of UNC-1 in the cyto-protection mechanisms of gastric mucosa is suggested by the finding that UNC-1 enhances cyto-protective bicarbonate and mucous production *(Flemström and Säfsten, 1994)* and induces prostaglandin E2 production in the GIT; which play a key role in maintenance of the GIT integrity and repair of gastric mucosal injury and ulcer healing *(Slomiany and Slomiany, 1991)*.

In the present study, we found that UNC-1 was present in the epithelial and mucous secreting cells in *H. pylori* positive specimens. UNC-1 was significantly decreased in patients with *H. pylori* positive gastritis in comparison with *H. pylori* negative patients which is in agreement with *Chatazki et al. (2003)*. There was also significant elevation of immunoreactive UNC-1 after *H. pylori* eradication in *H. pylori* positive patients in comparison to its level before eradication as previously reported by *Chatazki et al. (2003)* and *Testanis et al. (2005)*.

The correlation analysis clearly demonstrated a significant negative correlation between the level of immunoreactive UNC-1 and grading of gastritis

as assessed by the activity and inflammatory scores suggesting that regression of gastritis resulted in simultaneous increase in immunoreactive UNC-1 expression. As regression of gastritis resulted in decrease in the number of inflammatory cells, this explains the simultaneous increase in UNC-1 by the parietal cells of gastric mucosa. Based on these findings we can conclude that UNC–1 production is directly related to the defense mechanisms activated locally to protect gastric mucosa from noxious stimuli and it is involved in the local anti-inflammatory processes in the stomach in agreement with *Chatazki et al. (2003)*.

This study showed that the mucosal level of IL-1β was significantly higher in *H. pylori* positive than in *H. pylori* negative specimens in agreement with previous studies *(Noach et al. 1994 and Yamaoka et al. 1996)*. Our study demonstrated significant positive correlation between the level of IL-1β and grading of gastritis (activity and chronicity) suggesting that regression of gastritis resulted in simultaneous decrease in IL-1β content. Our results came in concordance with *Robert et al. (1991a & 1991b)* and *Yamaoka et al. (1997)* who suggested that IL-1β acts mostly by stimulating gastric prostaglandin synthesis. They proposed that the stomach possesses IL-1 receptors that were probably located on parietal cells, on prostaglandin producing cells, on smooth muscle cells (responsible for gastric emptying), and on as yet unidentified cells involved in gastric cyto-protection. Wolfe and Nompleggi *(1992)* estimated that on molar basis, IL-1β is 100 times more potent than both the prostaglandins and the proton pump inhibitor omeprazole and 6000 times more potent than cimetidine in inhibiting acid secretion.

Schepp et al. *(1998)* reported that the anti-secretory effect of IL-1 may contribute to hypoacidity secondary to acute *H. pylori* infection or during chronic colonization by *H. pylori* of the fundic gastric mucosa. The pro-inflammatory cytokine IL-1β directly influences the physiological events within the stomach that may become activated when the system is challenged by bacteria such as *H. pylori*. This pro-inflammatory effect of IL-1β contributes to the defense against pathogens and its acid inhibitory and cyto-protective effects contribute to the healing process following challenge to the integrity of the mucosa by *H. pylori* infection *(Saperas et al. 1992)*.

H. pylori eradication therapy has significantly reduced IL-1β immunostaining expression in *H. pylori* positive patients in the current study. This was explained previously by *El-Omar et al. (2000)* who found that eradication of *H. pylori* infection leads to permanent cure of ulcer diathesis as removal of the bacteria leads to down-regulation of the pro-inflammatory cytokines such as IL-1β and reversal of the hormonal abnormalities that cause acid hyper-secretion. As IL-1β is produced by PNC and MNC T helper (Th) lymphocytes *(Dinarello, 1996)*, this explains the significant decrease of Il-1β after *H. pylori* eradication in the present study.

This study showed that *H. pylori* positive cases had more extensive epithelial staining with IL-10 than *H. pylori* negative cases, reflecting more widespread epithelial degeneration but the difference was not significant in the current study in agreement with *Bodger et al. (2001)*. On the contrary, *Yamaoka et al. (1997)* reported that the level of mRNA expression of IL-10 was increased in *H. pylori* positive relative to *H. pylori* negative specimens using RT-PCR. They suggested that the discrepancies between mRNA and protein levels may be due to blockage of the pathway from mRNA to protein expression, degradation of mRNA prior to translation into protein, or simply the fact that the amount of mRNA did not exceed the threshold required for protein production.

IL-10 is a potent anti-inflammatory and immune-regulatory cytokine with a variety of biological effects, including inhibition of many

of the effector functions of phagocytes, such as the synthesis of pro-inflammatory cytokines e.g. TNF-α, IL-6 and IL-8 *(Fiorentino et al. 1991)*. A role for IL-10 in "damping down" the immune response to *H. pylori* infection is supported by the observation of the rapid development of severe hyperplastic gastritis in *Helicobacter felis* infected IL-10 (-/-) knockout mice *(Berg et al, 1998)*. Secretion of IL-10 might damp down the immune response to infection, limiting tissue damage but also potentially contributing towards the failure of the immune response to clear the organism *(Bodger et al. 1997)*.

We found that IL-10 was present in the surface epithelium, glandular epithelium and PMCs in agreement with *Bodger et al. (2001)* and *Ana et al. (2005)*. Despite the evidence that IL-10 expression/secretion by the whole gastric biopsies, *(Yamaoka et al. 1996 and Bodger et al. 1997)*; there was no report of *in situ* localization of the cellular sources of its production. Although originally described as a product of Th 2 lymphocytes, IL-10 is produced by a variety of cell types, notably mononuclear phagocytes *(Bodger et al. 1997)*. Present evidence suggests that a Th1 response predominates in *H. pylori* infection *(D'Elios et al. 1997)*, such that the production of IL-10 by Th 2 cells is unlikely to be a major source in gastritis *(Bodger et al. 2001)*.

We found that the mucosal level of IL-10 was not correlated with the activity score in agreement with *Bodger et al. (2001)* but correlated with the inflammatory score contrary to *Bodger et al. (2001)* too. This may be explained by the production of IL-10 by MNC and the role of IL-10 as an anti-inflammatory cytokine.

The present study showed that *H. pylori* eradication therapy has significantly reduced IL-10 immunostaining expression in *H. pylori* positive patients in agreement with *Fiorentino et al. (1991)*. As IL-10 produced by mononuclear cells; Th lymphocytes *(Dinarello, 1996),* this explains the significant decrease of IL-10 after *H. pylori* eradication in the present study.

There was no correlation between IL-1β and IL-10 but in the present study, nine specimens stained positively for IL-1β also showed strong IL-10 staining. On the other hand, the degree of staining of IL-10 was higher in *H. pylori* positive specimens with lower degree of IL-1β staining than in *H. pylori* positive specimens overall. IL-10 is thought to have a strong Down-regulatory effect on pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α that may explain this phenomenon. When the mucosal levels of these pro-inflammatory cytokines exceed a certain threshold, the production of IL-10 protein is also increased and the production of these pro-inflammatory cytokines may be reduced via the down regulatory effects of IL-10. Due to this complicated cytokine network, it may be very difficult to analyze the production of IL-10 by one point examination of biopsy specimens in agreement with *Yamaoka et al. (1997)*.

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الملخص العربي

اليوروكورتين والسيتوكينات في جدار المعدة: العلاقة بين النشاط الإلتهابي قبل وبعد القضاء على الإصابة بالبكتيريا الحلزونية هليكوباكتر بيلوري طبيا

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الخلفية والأهداف: هناك جدل عما إذا كانت غالبية إصابات التهاب المعدة المخاطي تحدث كنتيجة مباشرة للبكتيريا أو نتيجة لتلف الخلايا الناتج عن وسطاء الإستجابة الإلتهابية المناعية الوفيرة. وقد ظهر اليوروكورتين مؤخرا كوسيط للردود المعدية المعوية للمؤثرات الضارة. ويلعب الانترليوكين واحد بيتا والانترليوكين عشرة دورا هاما في التهاب المعدة الناجم عن الإصابة بالبكتيريا الحلزونية. وقد هدفنا إلى تحديد تعبير اليوروكورتين والانترليوكين واحد بيتا والانترليوكين عشرة الهستوكيميائي المناعي في خزعات منظار المعدة لتوضيح مشاركتها المحتملة في الإستجابة الإلتهابية المحلية قبل وبعد استئصال الإصابة بالبكتيريا الحلزونية طبيا.

طريقة البحث: أخذت خزعات المعدة من ٧٢ مريضا يشكون من أعراض عسر الهضم. وتم فحص العينات لتحديد التهاب المعدة ووجود أو عدم وجود بكتيريا وفحص تعبيراليوروكورتين والانترليوكين واحد بيتا والانترليوكين عشرة الهستوكيميائي المناعي.

النتائج: وقد كان وجود بكتيريا ايجابيا في ٧٢/٣٩ من الحالات وكان هناك اختلافا ذو دلالة إحصائية في درجة نشاط والتهاب المعدة وتعبيري اليوروكورتين والانترليوكين واحد بيتا بين عينات المرضى الإيجابية والسلبية للبكتيريا الحلزونية. وقد قلل العلاج بشكل كبير من نشاط ودرجة التهاب المعدة وتسبب في زيادة في تعبيراليوروكورتين وخفضا في تعبيري الانترليوكين واحد بيتا والانترليوكين عشرة في عينات المرضى إيجابية البكتيريا.
وكانت هناك علاقة سلبية كبيرة وإيجابية وليس هناك علاقة بين تعبيراليوروكورتين والانترليوكين واحد بيتا والانترليوكين عشرة على التوالي وبين نشاط التهاب المعدة.

الخلاصة: يشارك اليوروكورتين في العملية المضادة للإلتهابات المحلية النشطة في المعدة وقد يكون ذو صلة في علاجها سريريا. ويعتبر الانترليوكين واحد بيتا علامة على حدوث الالتهابات بوصفه يزداد كلما زادت شدة التهاب المعدة وينخفض بشكل ملحوظ بعد العلاج.