Expression of Cyclooxygenase-2 (COX-2) and Interleukin-8 (IL-8) in *H. pylori* Gastritis; a Study on Diagnostic Methods and Pathological Effects of *H. pylori* Infection in Dyspeptic Patients

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ABSTRACT

Background *H. pylori infection causes diverse clinical outcomes, including dyspepsia, peptic ulceration, gastric carcinoma and mucosa associated lymphoid tissue (MALT) lymphoma. Up-regulation of COX-2 has been observed in H. pylori gastritis in response to inflammatory cytokines. IL-8 is a major activator for neutrophils which contribute to mucosal damage in* H. pylori *infected patients. A wide-spread use of non-invasive simple diagnostic method is indicated for diagnosis and follow-up of H. pylori infection.* ***Aims:*** *Assessment of COX-2 and IL-8 immuno-expression in gastric mucosa in correlations to H. pylori infection in patients suffering from dyspepsia and evaluation of different available diagnostic modalities for detection of H. pylori infection in Sohag city, Egypt.* ***Methods****: The study included 62 patients complaining of dyspepsia. Stool samples were examined for detection of H. pylori stool antigen (HpSA) using enzyme immunoassay (EIA). Antral endoscopical biopsies were taken for culture, and histopathological evaluation using Giemsa stain. Immunohistochemical expressions of IL-8 and COX-2 in tissue sections were evaluated.* ***Results****: H. pylori infection was detected in 42/62 (67.7%) of patients with dyspepsia by using the gold standard method (culture & Giemsa stain). The diagnostic accuracy of HpSA was 83.8% which made it a good non-invasive alternative for detection of H. pylori infection in our community. COX-2 was expressed in 90.5% of H. pylori positive and in 55% of H. pylori negative cases (P < 0.003). IL-8 was expressed in 83.3% of H. pylori positive and in 50% of H. pylori negative cases (P < 0.003).* ***Conclusions****: Wherever endoscopy is not indicated, HpSA EIA is a non invasive, rapid, easily performed, reliable method for diagnosis of H. pylori infection. H. pylori infection causes enhancement of COX-2 and IL-8, and this may have a role in the progression of gastritis to more advanced lesions.*

Keywords: *H. pylori gastritis, H. pylori stool antigen (HpSA) enzyme immunoassay (EIA) COX-2 and IL-8*

Abbreviations:
Cyclooxygenase-2 (COX-2), Interleukin 8 (IL-8), *H. pylori* stool antigen (HpSA), enzyme immunoassay (EIA), prostaglandins (PGs), prostacyclin (PGI2) and thromboxane A2 (TXA2), reactive oxygen species (ROS), T helper 1 (Th1), gastrointestinal (GI), hematoxylin and eosin (H&E), phosphate buffered saline (PBS), normal goat serum (NGS), negative and positive predictive values (PPV & NPV).

INTRODUCTION

In 1984, **Marshall and Warren**[(1)] reported the discovery of a bacterium, which was subsequently named *Helicobacter pylori; H. pylori*[(2)], whose habitat was the human gastric mucosa. *H. pylori* infection in the stomach induces mucosal inflammation and oxidative stress that leads to diverse clinical outcomes such as gastritis, peptic ulcer and gastric cancer. Chronic gastric inflammation developing after *H. pylori* infection is responsible for dyspeptic symptoms relevant to gastritis[(3)]**.** *H. pylori* is well recognized also as a class I carcinogen because long-term colonization by this organism can provoke chronic inflammation and atrophy, which can further lead to malignant transformation[(4,5)]**.** Since its cure prevents peptic ulcer disease, chronic gastritis, gastric carcinoma and possibly MALT lymphoma, the accurate detection of *H. pylori* is essential for the management of patients and for eradication of the bacterium following treatment.

H. pylori infection could be diagnosed either by non invasive methods that reveal the presence of *H. pylori* as urea breathe test, and serology or invasive procedures requiring endoscopic biopsy of the gastric mucosa for

histopathological analysis, culture, or rapid urease test[6]. Non invasive tests facilitates epidemiological research, decreases the overall endoscopy workload and accordingly financial savings. In addition, non invasive techniques can assess *H. pylori* status after treatment, obviating the need for re-endoscopy. Now, direct detection of the *H. pylori* antigens in stool samples has been developed. Studies using EIA have shown that it is a reliable tool for non invasive diagnosis of *H. pylori* infection and for epidemiological studies [7].

H. pylori induces a strong and complex immune response in the gastric mucosa, both humoral and cellular, which nevertheless fails to clear the infection and may even contribute to immunopathology[8,9]. The exact mechanisms by which the *H. pylori*-induced immune response contributes to gastrointestinal mucosal damage are still not clear. At this time, it is not known whether this immune response is protective or whether it contributes to the pathogenesis of *H. pylori*-associated diseases.

Cyclooxygenase (COX) is a rate-limiting enzyme in the synthesis of prostaglandins (PGs). It catalyses the conversion of arachidonic acid to PGG2, then to PGH2 which is subsequently converted to various physiologically active prostanoids, including PGE2, PGD2, prostacyclin (PGI2) and thromboxane A2 (TXA2) by the relevant enzymes in a variety of cell types. At least 2 isoforms of COX have been identified so far[10,11].

COX-1 is expressed constitutively in many tissues and in the normal esophageo-gastro-colonic mucosa. PGs produced by COX-1 mediate the "housekeeping" functions such as cytoprotection. In contrast, COX-2 is not detected in most normal tissues, but its expression is rapidly induced by both inflammatory and mitogenic stimuli resulting in increased synthesis of PGs in the inflamed and in the neoplastic tissue [11, 12].

COX-2 was found to be expressed in the mucosa of *H. pylori*-induced gastritis. In chronic *H. pylori* infection, overexpression of COX-2 is probably induced by inflammatory cytokines, growth factors, especially gastrin and reactive oxygen species (ROS) leading to mutagenesis and subsequent metaplasia, dysplasia and cancer formation[11,12]. The precise mechanisms leading to the overexpression of COX-2 are still not fully understood. There is evidence that pro-inflammatory cytokines and different gastric mucosal growth factors could be involved in this process[11,13]. *H pylori* infection could induce gastric mucosal cell proliferation by COX-2 expression [14]. COX-2 gene expression was one of the factors mediating the progress from *H pylori* gastritis to pre-carcinoma and even gastric carcinoma [15, 16].

H. pylori could induce the production of inflammatory mediators such IL-8, IL-6, and TNFa[17] and secondary high COX-2 expression which caused gastric mucosal lesions. These mediators could directly or indirectly damage the surface epithelial cells[18]. Among these mediators, IL-8 plays a crucial role, it is expressed in injured mucosa after *H. pylori* infection and increases mucosal injury. It is a major activator and chemokine for neutrophils which contribute to mucosal damage[19,20]. IL-8 significantly augments T helper 1 (Th1) immune response by inducing pro-inflammatory cytokines such as TNF-α, interferon-γ, and IL-1β secretion[21,22]. Hence, IL-8 is an agent that could modulate these key events; this might propose an effective strategy to prevent *H. pylori*-induced pathological disorders [20, 23].

Aims:

This study aims to assess the immunohistochemical expression of COX-2 and IL-8 in the gastric mucosa in correlation to *H. pylori* gastritis and evaluation of different available diagnostic modalities for detection of *H. pylori* infection in patients suffering from dyspepsia visiting the Outpatient Clinic of Internal Medicine Department, Sohag University, Egypt, to determine whether simple non-invasive method as HpSA could be a valuable test for diagnosis of *H. Pylori* infection in a symptomatic patient.

PATIENTS & METHODS

This study was performed in collaboration between Internal Medicine, Pathology and Biochemistry Departments of Sohag University and Microbiology and Immunology Department, Assiut University during the period from March 2005 to July, 2005. The study included 66 selected patients (24 males & 38 females) with age range between 20 years to 60 years with a mean age SD of 32.7 ± SD 9.7 years. Patients complained of symptoms suggestive of upper gastrointestinal (GI) diseases including recurrent upper epigastric pain, heart burn, vomiting, distension and anorexia. All patients gave an informed consent before inclusion in the study and the study was approved by the Local Ethics Committee.

All patients were subjected to full clinical history and examination. All were satisfied the following eligibility criteria, age above 18

years, no administration of antibiotic or proton pump inhibitors in the two months prior to the study, no administration of *H. pylori* eradication treatment in the last 12 months. Patient with active GI bleeding were excluded from the study. Liver and kidney function tests and abdominal sonogrophy was done to exclude hepatobiliory and/or renal diseases.

Upper GI endoscopy was done and multiple antral and corporal gastric biopsies were processed for culture sensitivity studies and for histopathological examination. Stool samples were collected from all patients for detection of HpSA using polyclonal EIA kit. Stool samples were stored at -20°C until the analysis. *H. pylori* infection was identified if culture and/or histopathology; Gemisa stain yielded positive results [24].

Culture and antibiotic susceptibility testing:

The gastric biopsy was kept at 4°C in 5ml sterile 0.9% NaCl and was examined within 24 hours. Each biopsy was cut into small pieces and homogenized in sterile Petri dish. The specimens were cultured on Columbia agar plates supplemented with 5% sheep blood and *H. pylori* selective supplement vials (Oxoid). The vial contains vancomycin in (10 mg/L), cefuslodin (5 mg/L), trimethoprim (5 mg/L) and amphotericin B (5 mg/L). The inoculated plates were placed in an anaerobic jar together with a gas generating sachet (Oxoid) to generate a microaerophilic environment (10% CO_2, 5% O_2 and 85% N_2) and incubated for 5-10 days at 37°C. *H. pylori* microorganisms were identified on the basis of characteristic colony morphology, typical appearance on Gram staining and strong positive reactions with oxidase, catalase and urease tests. Isolates were preserved in *H. pylori* special peptone broth media containing 10% glycerol at -80°C.

Determination of *H. pylori* antigens in stool samples

H. pylori antigens in stool samples were detected by using Astra Diagnostic *H. pylori* antigen quantitative EIA commercial kit (Milana, Italy). An extraction kit for *H. pylori* antigen from stool was initially used by applying the dipstick to collect 6-7 mm stool samples, placed in stool dispenser buffer containing tubes, vortexed for 30 seconds. The tips of the tubes were broken and 3 drops were applied into appropriate microwell of the Astra HP Ag kit coated with polyclonal capture anti *H. pylori* antibody. Hundred ml of HRP peroxide conjugated polyclonal anti *H. pylori* antibody was added into the wells and incubated for 1 hour at 37°C. After 4 proper washes with saline solution with added thimerosal and surfactant; 200 ml of TMB substrate were added to the plate, incubated for 10 minutes at 37°C in the dark. Finally the reaction was stopped by 100 ml 0.3 M sulphuric acid solution (Stop solution). Positive and negative controls were also built into the test.

The test was measured using EIA reader at 450/630 mm within 15 minutes of adding stop solution. The cut-off was calculated as the OD of negative control + 0.150 according to the manufacturer's instruction. The cut-off OD value was 0.342. Samples with OD values more than Co + 10% (0.376) were considered positive, samples with OD values within ± 10% of cut-off (0.376-0.308) were considered borderline and were repeated for confirmation and samples with OD values less than Co – 10% (0.308) were considered negative.

Histopathology

Three biopsy specimens were obtained from each of the fundus, corpus, and antral regions. Biopsy specimens were fixed in 10% diluted formalin, embedded in paraffin, 5 µm tissue sections were made and stained by hematoxylin and eosin (H&E) to evaluate the presence or absence of gastritis, activity and/or chronicity using Sydney System for classification of gastritis[25]. Modified Giemsa stain was used on subsequent levels of the same blocks by the method described by **Gray et al.,[26].** The presence of *H. pylori* was detected by Giemsa staining as spiral bacilli in the gastric mucosa attached to mucin using bright field light microscope.

Immunohistochemistry

Since the *H. pylori* positivity is high in the antral region, only specimens from the antral region were used for immunohistochemical staining. Serial 4 µm tissue sections on silanized glass slides were stained using primary rabbit polyclonal antibody to COX-2 (Cat # RB-9072-P0, 0.1ml, LabVision Corporation) and mouse monoclonal antibody to IL-8 (Cat # C-19, sc1269, Santa Cruz Biotec). Tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked using peroxidase blocking reagent (Cat # TP-012-HD, LabVision Corporation). Unmask the antigen sites by immersing the slides in sufficient amounts of antigen retrieval solution (10 mmol sodium citrate buffer, pH 6.0) was done. Sections were microwaved for 10-15 min, allowed to cool down for 20 min, washed in distilled water, then in phosphate buffered saline (PBS, pH 6.0). Tissue sections were incubated in normal goat serum (NGS) to block the nonspecific interactions.

Tissue sections were incubated for half hour at room temperature with 1/200 COX-2 antibody and overnight at 4°C in a humid chamber with 1/100 IL-8 antibody. The resulting immune-complex was detected by a universal staining kit (Cat # TP-012-HD, LabVision Corporation). Tissue sections were treated with biotinylated goat anti-polyvalent, and then peroxidase-labelled streptavidin was applied for 10-15 min at room temperature, rinsed in PBS, incubated with 14-diaminobenzidine and 0.06% H_2O_2 for 5 min and counter-stained in Myer's Hematoxylin. Tissue sections were washed in tap water, dehydrated in alcohol, cleared in xylene, left to dry, then mounted with Canada balsam, and cover slipped.

Positive controls were prepared from colon carcinoma and from skin for detection of COX-2 and IL-8 respectively. ***Negative controls*** were done by omitting the primary antibody from the staining procedure. The positive and negative controls were consistently immunoreactive and lacking reactivity. This confirms the validity of staining results.

For simplicity we divide immunostaining positivity of COX-2 and/or IL-8 staining into 3 grades; negative (grade 0) if faint focal, weak if weak diffuse or moderate focal staining (grade I), and strong if strong focal or diffuse staining or moderate diffuse (grade II) is present all over the slide.

Statistical methods;

Diagnostic accuracy, sensitivity, specificity, positive and negative predictive values were determined for the EIA stool antigen test for detecting *H. pylori* infection against the gold standards methods of diagnosis; culture and histopathology among all tested patients. Fisher's exact, Chi square (X^2), and analysis of variance (ANOVA) were used for analysis of immunostaining results using Statistical Package of Social Science (SPSS, version 10 SPSS Inc., Chicago, USA) and P value less than 0.05 was considered significant.

RESULTS

Histopathology and culture sensitivity:

Figure (1; A-F): shows the results of histopathological; H&E (A) and Gimesa stain (B) and immunostaining of COX-2 and IL-8 (C-F) in *H. pylori* positive and *H. pylori* negative gastritis.

Examination of H&E stained slides revealed mildly active gastritis in 10/62 (16.1%) cases and moderately active gastritis in 8/62 (12.9%) cases and chronic gastritis in 44/62 cases. Giemsa stain revealed positive *H. pylori* in 14/42 (33.3%) of active gastritis, whereas 28/42 (66.7%) of *H. pylori* positive patients were presented by chronic gastritis as shown in Table (1).

Among the participants 40/62 (64.5 %) were *H. pylori* positive by culture alone, 36/62 (58.1%) were positive by Gemisa stain and 34/62 (54.8%) were positive by both culture and Giemsa stain. The total number of *H. pylori* positive cases was 42/62 (67.7 %). Table (2) displays the correlations between the type of gastritis and *H. pylori* positivity using the gold standard methods; culture and Giemsa stain. Primary resistance to metronidazole, clarithyromycin, amoxacillin and tetracyclines was found in 100%, 62.5%, 60% and 12.5% respectively.

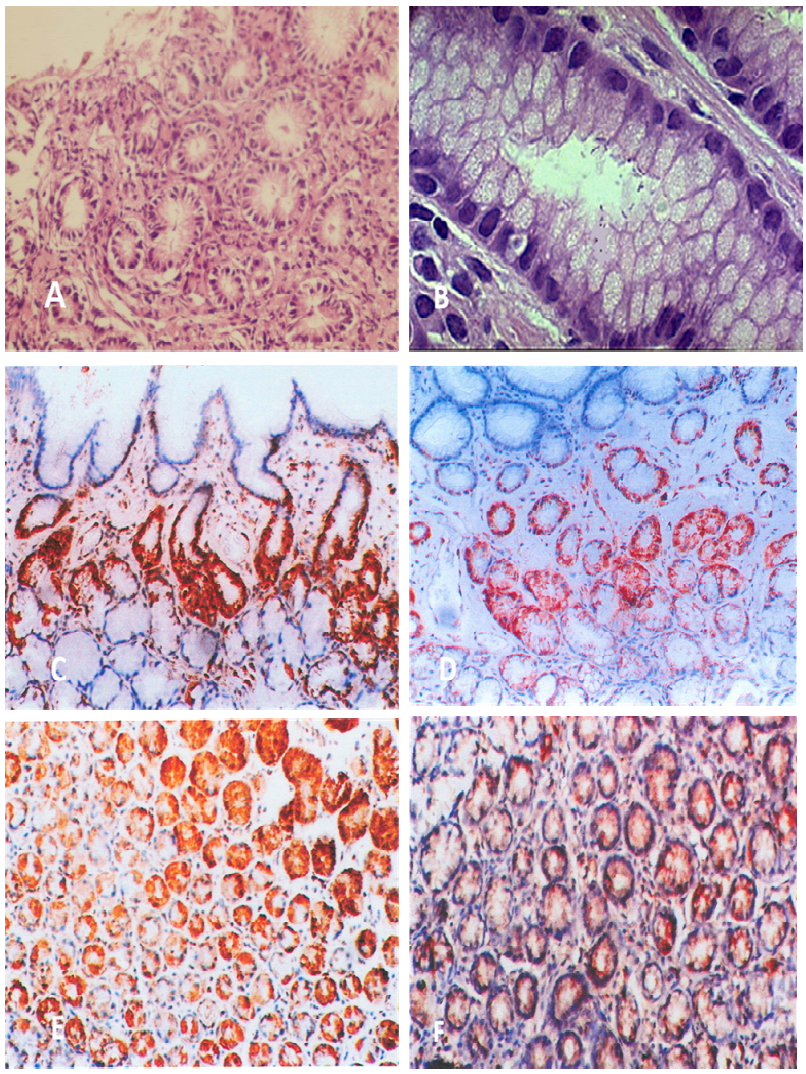

Figure (1): *H. pylori* positive gastritis H&E stain (A), Gimesa stain (B), strong COX-2 expression (C) & IL-8 (E) and *H. pylori* negative gastritis; weak COX-2 expression (D) & IL-8 (F). Magnifications: X400 (A), X1000 (B), X200 (C-F).

Table (1): Correlations between type of gastritis and *H. pylori* positivity using the gold standard method (culture & Giemsa stain)

Histopathological results	***H. pylori* positive**	***H. pylori* negative**	**Total**
Mild gastritis	6/10	4/10	10
Moderate gastritis	8/8	0/8	8
Severe gastritis	----	----	----
Chronic gastritis	28/44	16/44	44
Total	42/62	20/62	62

Table (2): *H. pylori* infection in gastric biopsy using the gold standard methods (culture and Giemsa stain)

***H. pylori* infection**	**Culture/Histopathology**	**No. of patients/Total**	**(%)**
Positive 42/62	Positive / Negative	6/62	9.7
	Positive / Positive	34/62	54.8
	Negative / Positive	2/62	3.3
Negative 20/62	Negative / Negative	20/62	32.3

HpSA test:

The HpSA test was positive in 36/42 of *H. pylori* positive patients as determined by the standard methods, and negative in 16/20 of *H. pylori* negative patients. The sensitivity and specificity of HpSA test were 85.7 % and 80 % respectively while the negative and positive predictive values (PPV & NPV) were 72.7 % and 90 % respectively. The overall diagnostic accuracy was 83.8%. Table (3) shows the correlation between the standard methods of *H. pylori* detection versus HpSA EIA status.

Table (3): Comparison of HpSA EIA versus standard methods (culture and Giemsa positive stain) for detecting *H. pylori* infection among all studied patients

Standard methods	**HPSA EIA**		Total
	Positive	**Negative**	
***H. pylori* Positive**	36	6	42
***H. pylori* Negative**	6	16	22
Total	42	20	62

Diagnostic accuracy: 83.7%, Sensitivity: 85.7%, Specificity: 80%, PPV: 90%, NPV: 72.7%.

Results of immunohistochemistry:

COX-2 was localized to the gastric epithelial cells in the deep antral glands and in the monocytic cells in the lamina propria in both *H. pylori* positive and negative cases in the current study. IL-8 immunostaining was expressed in the superficial epithelium and mononuclear inflammatory cells; lymphocytes, monocytes and plasma cells infiltrating the lamina propria of the stomach. Immunostaining positivity of COX-2 and/or IL-8 appeared as granular cytoplasmic brown staining in the epithelial cells in the gastric mucosa that was graded according the surface area and the intensity of the stain into grades 0, + I and + II.

Table (4-6) and Figure (2, 3) showed that among *H. pylori* positive cases 38/42 (90.5%) were COX-2 positive whereas among *H. pylori* negative cases 11/20 (55%) were COX-2 positive and this difference was highly significant ($P < 0.003$). COX-2 was strongly positive in 30/38 (90.5%) *H. pylori* positive cases, however it was strongly positive in 4/20 (20%) *H. pylori* negative cases and the difference in the grade of COX-2 staining between *H. pylori* positive and *H. pylori* negative cases was highly significant ($P<0.000$). In *H. pylori* positive cases there was no statistical difference in COX-2 expression between acute and chronic gastritis ($P < 0.456$).

Table (7-9) and Figure (4, 5) showed that among *H. pylori* positive cases 35/42 (83.3%) were IL-8 positive, whereas among *H. pylori* negative cases 10/20 (50%) were IL-8 positive and this difference was highly significant ($P < 0.012$). IL-8 was strongly positive in 29/35 (82.9%) *H. pylori* positive cases, however it was strongly positive in 5/20 (25%) *H. pylori* negative cases and the difference in the grade of staining of IL-8 between *H. pylori* positive and *H. pylori* negative cases was highly significant ($P < 0.004$). In *H. pylori* positive cases there was no statistical difference in IL-8 expression between acute and chronic gastritis ($P< 0.569$).

Table (4): *H. pylori* positivity versus COX-2 immunostaining expression

COX-2 expression	**Total No./%**	**COX-2 +ve**	**COX-2 -ve**	**X2/p value**
H. pylori +ve	42/62 (67.7 %)	38/42 (90.5%)	4/42 (9.5%)	
H. pylori -ve	20/62 (32.3%)	11/20 (55%)	9/20 (45%)	P < 0.003
Total	62/62 (100%)	49/62 (79%)	13/62 (21%)	

Table (5): *H. pylori* positivity versus COX-2 immunostaining expression

COX-2 expression	**Total No./%**	**H. pylori +ve**	**H. pylori -ve**	**X2/p value**
Grade 0	13/62 (21%)	4/42 (9.5%)	9/20 (45%)	
Grade I	15/62 (24.2%)	8/42 (19.1%)	7/20 (35%)	P < 0.000
Grade II	34/62 (54.8%)	30/42 (71.4%)	4/20 (20%)	
Total	62/62 (100%)	42/62(67.7 %)	20/62 (32.3%)	

Table (6): COX-2 expression in acute and chronic gastritis

***H. pylori* gastritis**	**COX-2 positive**	**COX-2 negative**	**P value**
Acute gastritis	12/14	2/14	P < 0.456
Chronic gastritis	26/28	2/28	
Total	38/42	4/42	

Table (7): *H. pylori* positivity versus IL-8 immunostaining expression

IL-8 expression	**Total No./%**	**IL-8 +ve**	**IL-8 -ve**	**X2/p value**
H. pylori +ve	42/62 (67.7 %)	35/42 (83.3%)	7/42 (16.7%)	P < 0.012
H. pylori -ve	20/62 (32.3%)	10/20 (50%)	10/20 (50%)	
Total	62/62 (100%)	45/62 (72.6%)	17/62 (27.4%)	

Table (8): *H. pylori* positivity versus IL-8 immunostaining expression

IL-8 expression	**Total No./%**	**H. pylori +ve**	**H. pylori -ve**	**X2/p value**
Grade 0	17/62 (27.4%)	7/42 (16.7%)	10/20 (50%)	P < 0.004
Grade I	11/62 (17.7%)	6/42 (14.3%)	5/20 (25%)	
Grade II	34/62 (54.8%)	29/42 (69%)	5/20 (25%)	
Total	62/62 (100%)	42/62(67.7 %)	20/62 (32.3%)	

Table (9): IL-8 expression in acute and chronic gastritis

***H. pylori* gastritis**	**IL-8 positive**	**IL-8 negative**	**Total**
Acute gastritis	11/14	3/14	P< 0.569
Chronic gastritis	24/28	4/28	
Total	35/42	7/42	

DISCUSSION

H. pylori is one of the most widespread infections in human worldwide. It persistently infects up to 50% of the world's population **(27)**. Higher prevalance is found in developing countries. Many researchers recommended test and treat strategy for young patients with dyspepsia without alarm symptoms **(28, 29)**.

Since the discovery of *H. pylori*, several invasive and noninvasive methods diagnostic methods have become available for determining the presence of *H. pylori* infection**(30)**. Noninvasive tests are serological methods, Urea breath test, and bacterial DNA sequences or bacterial antigen detection in stool by the HpSA test**(31)**. In contrast culturing *H. pylori* for diagnosis of infection requires gastric biopsy obtained by the invasive gastroduodenoscopy**(32)**. Culture methods require an incubation period of at least 4-7 days. *H. pylori* is also a fastidious microorganism and is affected by environmental conditions **(33)**.

Noninvasive tests are preferred because of their simplicity and the ability to provide test results within a few minutes after administration, in a physician's office. The urea breath test was the test of choice when the test and treat strategy was implemented, and its availability resulted in the reduction of the number of endoscopy referrals among patients below 40 years of age in a study by **Sreedharan et al.,(34)**. The Urea breath test is currently regarded as the best non invasive diagnostic method for detecting *H. pylori* infection, even for monitoring the efficacy of treatment. However this method is not widely available in our locality.

Serology requires only venipuncture, but is an unreliable test for the diagnosis of *H. pylori* infection and may or may not revert to negative after eradication of the bacteria, also positive results do not necessarily indicate current infection **(35,36)**.

In this study the sensitivity and specificity of the HpSA test were 85.75% and 80% respectively. Several studies reported a wide range of specificity and sensitivity values with a sensitivity being as low as 72.7%, 63% and 58% respectively **(37, 38)**.

In contrast specificity was less than reported by others being over 90% in the study of **Koletzko et al.(24)**. Low sensitivity in the current study may be due to difficulty of obtaining polyclonal antibodies of constant quality and the variability of the cut-off values of the test assays. In the monoclonal version of the assay, **Koletzko et al.(24)** reported that the difference between positive and negative results was valuable with a diagnostic accuracy of

97.8%. This was attributed to the cut-off values as no gray zones was present because positive samples gave values far above and negative samples gave values clearly below the cut-off values.

HpSA test may be of particular use in pediatric and old aged groups where noninvasive tests are preferred. Moreover EIA had several advantages over other diagnostic tests requiring endoscopic biopsies. Firstly, patients are not required to attend hospitals as stool samples can be transported to the laboratory. Secondly, neither an expensive instrumentation as in case of upper GI endoscopy, nor an expertise as in case of PCR or culture is needed to perform the EIA test. However investigators cautioned against the complete avoidance of endoscopy due to the risk of missing of some important pathological findings[37]. Further studies are required to assess and compare the performance of HpSA EIA monoclonal and polyclonal versions for the non invasive diagnosis of dyspepsia.

In this study, *H. pylori* was detected in 42/62(67.7%) of biopsy specimens by the standard methods; of which 14/42 (33.3%) patients were presented with active gastritis and 28/42 (66.7%) patients were presented with chronic gastritis in agreement with **Demiray et al.,**[39] **Abdullah et al.,** [40] **and Jemilohun et al.,**[41] who found *H. pylori* in 68.2%, 68% and 64% of the studied patients respectively. However, our result was slightly lower than that of **Kang et al.,**[42] who detected *H. pylori* in 873 of 1197 patients (73%). This difference may be due to the larger number of the studied patients in their study. Our results were slightly higher than that of **Bruden et al.,**[43] who found that among the studied population 53% were positive for *H. pylori*. The high incidence of infection in the current study can be explained by the fact that the investigated population was symptomatic patients.

H. pylori infection induces active inflammation with neutrophilic infiltration and elicits chronic inflammation with lymphocytes, macrophages/monocytes and plasma cells infiltration in the lamina propria of the gastric mucosa[44,45], yet only a minority of infected individuals develops peptic ulcer disease, atrophic gastritis, or gastric malignancies. The severity, progression, and consequences of *H. pylori* infection have been shown to depend on the host genetic background and in particular on gene polymorphisms affecting the host immune response [46].

COX-2 inhibitors suppressed prostaglandin synthesis and aggravated mucosal damage. In human stomach, COX-1 appears to be the predominant source of prostaglandins despite the fact that COX-2 is up-regulated in *H. pylori* gastritis. There are conflicting data on whether *H. pylori* alters the risk of ulcer in patients receiving COX-2 inhibitors. The functional significance of COX-2 in human gastric ulcer is unknown [47].

COX-2 is induced in numerous processes such as cellular growth, differentiation, inflammation and tumorogenesis[48]. *H. pylori* infection causes chronic gastritis and induces COX-2 expression[49]. All chronic gastritis cases displayed positive epithelial COX-2 expression with only 25% positivity showed stromal expression in the study of **Samaka et al.,**[48]. COX-2 appears to be involved in gastric carcinoma progression as it promotes angiogenesis, suppresses apoptosis and facilitates invasion and metastasis[48,50].

Meyer et al., [51] suggests that COX-2 has an immunosuppressive role in *H. pylori* gastritis, which may protect the mucosa from severe injury, but may also contribute to the persistence of the infection. COX-2, deficiency suppresses *H. pylori*-induced cell proliferation[52]. Selective COX-2 inhibitors have been shown in numerous studies to exhibit strong chemo-preventive effect on the development of gastrointestinal cancers [12]. **Zhang et al.** [53] studied the effect of treatment with celecoxib, a selective COX-2 inhibitor following *H. pylori* eradication on COX-2 protein expression which was significantly increased in gastric precancerous lesions (atrophy, intestinal metaplasia and dysplasia, respectively) compared with chronic gastritis. They observed a significant improvement in precancerous lesions in patients who received celecoxib compared with those who received placebo ($P < 0.001$). No significant reduction in COX-2 or improvement in gastritis was found in subjects with eradication failure [49].

COX-2 inhibitors suppressed prostaglandin synthesis and aggravated mucosal damage. In the human stomach, COX-1 appears to be the predominant source of prostaglandins despite the fact that COX-2 is upregulated in *H. pylori* gastritis. There are conflicting data on whether *H. pylori* alters the risk of ulcer in patients receiving COX-2 inhibitors. The functional significance of COX-2 in human gastric ulcer is unknown [54].

Consistent with **Kim et al.,**[55] **and Bhandari et al.,** [56] COX-2 was localized to the gastric epithelial cells in the deep antral glands and in the monocytic cells in the lamina propria in both *H. pylori* positive and negative cases in

the current study. IL-8 immunostaining was expressed in the superficial epithelium and mononuclear inflammatory cells; lymphocytes, monocytes and plasma cells infiltrating the lamina propria of the stomach [56, 57].

There are conflicting reports on the expression of cyclooxygenase in *H. pylori* infection. In agreement with **Forones et al.,**[58], **Cho et al.,** [59] **and Erkan et al.,**[60], current study showed that *H. pylori* positive cases significantly expressed COX-2 staining more than *H. pylori* negative cases ($P < 0.003$). We found also that COX-2 was strongly expressed in *H. pylori* positive cases than in *H. pylori* negative cases ($P<0.000$). Similarly, COX-2 expression was significantly higher in the *H. pylori*-infected group in children in the study of **Kim et al.,** [55].

There was no statistical difference in COX-2 expression between acute and chronic gastritis ($P < 0.456$) in *H. pylori* positive cases consistent with **Ding et al.**[61]. In contrast, **Kim et al.**[51] reported that COX-2 expression was correlated with acute and chronic inflammation in children. **Samaka et al.**[48] reported that COX-2 expression was correlated with chronic gastritis, while **Fukuhara et al.**[62] found that there was a significant correlation between acute gastritis as assessed by neutrophil infiltration and COX-2 expression ($P<0.001$). This difference may be due to the lower number of acute gastritis in the present study.

There was a steady increase in COX-2 expression ongoing from normal mucosa to chronic active gastritis to intestinal metaplasia patients in the study of **Erkan et al.,**[60]. *H. pylori* infection induces COX-2 not only in inflammatory responses but also in gastric carcinogenesis[63]. There was also a stepwise increase in the expression of COX-2 as mucosal damage progressed from normal to gastritis to gastric ulcer in the study of **Bhandari et al.**[56]. A significant steady increase in COX-2 starting at atrophic gastritis to intestinal metaplasia and gastric carcinoma was reported by **Ding et al.,**[61]. This was confirmed by the findings of **Kim et al.**[64], **Cho et al.**[59] **and Erkan et al.**[60] who reported that COX-2 plays an important role in the stepwise process that eventually leads to gastric cancer.

The *H. pylori* eradication, failed to reverse the histological atrophy of the gastric mucosa but improved significantly the functional status of the atrophied mucosa, attenuated the expression of premalignant markers such as COX-2, raised the production of growth factors and diminished the release of proinflammatory cytokines[65]. Several prescriptions have been made for decreasing COX-2 levels resulting in attenuation of *H. pylori*-induced gastric lesions[64, 66].

IL-8 is one of the most crucial cytokine in the host inflammatory response to *H. pylori* infection and is a potent neutrophil chemoattractant[20]. The up-regulation of IL-8 in *H. pylori* infection may lead to free-radical generation and the release of proteolytic enzymes from activated neutrophils ultimately affecting mucosal integrity[67]. IL-8 and ROS, increased cell proliferation combined with inhibition of apoptosis as well as up-regulation of proliferation [13].

Current study showed that *H. pylori* positive cases revealed IL-8 expression more than *H. pylori* negative cases ($P < 0.003$) in agreement with **Yamaoka et al.,**[68], **Bontems et al.,**[69] **and Ziadi et al.,**[23]. Additionally, several studies investigating IL-8 have shown an association between the levels of this cytokine and *H. pylori* infection[70,71]. The difference in IL-8 production, as measured by immunohistochemistry, was much higher in *H. pylori*-infected adults in the study of **Bontems et al.,**[69] than in *H. pylori*-infected children in the study of **Lopez et al.,**[72]. In contrast to our findings, **Lopes et al.**[72] reported that the proportion of the epithelial IL-8 staining did not differ significantly between *H. pylori* positive and *H. pylori*-negative cases.

The current study revealed that in agreement with **Lopes et al.**[72], IL-8 expression in *H. pylori* positive cases was mostly strong in comparison with the weaker positivity in *H. pylori* negative cases ($P<0.004$). However, IL-8 was also detected in the epithelium of normal gastric mucosa in some studies[73,74]. We found that in *H. pylori* positive patients IL-8 expression showed no statistical difference comparing acute gastritis and chronic gastritis ($P< 0.569$).

We aimed in the present study to evaluate the influence of *H. pylori* on COX-2, and IL-8 expression. Our results suggest that the higher levels of IL-8, and COX-2 detected in gastric mucosa from *H. pylori* infected patients consistent with the study of **Bartchewsky et al.,**[75]. *H. pylori* infection might activate nuclear factor-kappaB (NF-kappaB), a transcriptional regulator of IL-8 and COX-2 and increased their levels leading to chronic gastritis and subsequent more advanced lesions [76].

In conclusion, our study confirmed the presence of high expression of COX-2 and IL 8 in patients with *H. pylori*-induced gastritis. As COX-2 is suggested to have a role in tumorigenesis, simple non-invasive method for

detection of *H. pylori* infection in symptomatized patient is recommended to be widely used as pre-treatment requisite in dyspeptic patients. In this study, we found that HpSA is of considerable accuracy. Further studies are recommended to elucidate whether *H. pylori* eradication alone is sufficient to normalize the high expression of COX-2 in gastric precancerous lesions or additional anti COX-2 must be used to decrease the risk of malignancy.

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التعبير المناعي-الهستو كيميائى للسيكلواوكسيجينيز-٢ والانترليوكين -٨ فى التهابات الغشاء المخاطى المبطن لجدار المعدة الناتج عن العدوى بميكروب الهليكوباكتر: دراسة لطرق التشخيص والتأثيرات المرضية لعدوى الهليكوباكترفى مرضى عسر الهضم

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مقدمة:
تسبب العدوى بميكروب الهليكوباكترأعراض عسرالهضم عن طريق الاصابة بالتهابات فى الغشاء المخاطى للمعدة أو قرحة المعدة أو الاثنى عشر وقد يتطور الأمر الى حدوث سرطان المعدة أو ليمفوما الأغشية المخاطية. وقد لوحظ فى العديد من التجارب السابقة وجود زيادة فى التعبير المناعى-الهستوكيميائى لبروتين السيكلوأكسيجينيز-٢وكذلك الانترليوكين-٨ فى أنسجة الغشاء المخاطى المبطن للمعدة المصابة بعدوى الهليكوباكتر ويعتقد الباحثون وجود علاقة بين هذه الزيادة وتطور الضرر فى الأنسجة لحدوث الأورام. لذلك فهناك حاجة لتوفير طريقة بسيطة (فى الحالات التى لا تستدعى الفحص بمنظار المرىء والمعدة) لتشخيص عدوى الهليكوباكتر فى مرضى عسر الهضم.
الهدف من الدراسة:
تقييم التعبير المناعى الهستوكيميائى لكل من السيكلوأكسيجينيز-٢ وللانترليوكين-٨ فى النسيج المخاطى لجدار المعدة المصاب بعدوى الهليكوباكتر فى مرضى عسر الهضم وكذلك تقييم الطرق المتوفرة لتشخيص تلك العدوى فى مرضى مدينة سوهاج بمصر.
طرق الدراسة:
اشتملت الدراسة على ٦٢ مريض يعانون من أعراض عسر الهضم والذين ترددوا على العيادات الخارجية لأمراض الباطنة بمستشفى سوهاج الجامعى. بعد أخذ موافقة المرضى تم أخذ عينة براز من كل مريض للكشف عن أنتيجين الهليكوباكتر فى البراز. تم عمل فحص بواسطة منظار المرىء والمعده لكل مريض وأخذ عدة عينات من المعدة حيث تم عمل مزرعة ميكروبية للهليكوباكتر, فحص باثولوجى بواسطة صبغة الهيماتوكسيلين والايوسين والفحص بواسطة صبغة الجيمسا لتحديد ميكروب الهليكوباكتر والفحص المناعى الكيميائى لتقييم السيكلوأكسيجينيز-٢ وللانترليوكين-٨
النتائج:
تم تشخيص وجود عدوي الهليكوباكتر فى مرضى عسر الهضم بالطرق التقليدية (المزرعة البكتيرية و/ او الفحص الباثولوجى) فى٧.٦٧% من مرضى عسر الهضم الخاضعين للدراسة. وأظهرت الدراسة الحالية أن دقة تشخيص العدوى بواسطة استخدام أنتيجين الهليكوباكتر فى البراز تساوى ٨٣.٨% مما يجعل هذه الوسيلة البسيطة ملائمة لتشخيص هذه العدوى فى مجتمعنا.
وقد أظهرت الدراسة وجود ارتفاع ذو دلالة احصائية فى التعبير عن السيكلوأكسيجينيز-٢ وكذلك الانترليوكين-٨ فى أنسجة الأغشية المخاطية المبطنة لجدار المعدة المصابة بعدوى الهليكوباكتر.
الخلاصة:
يعتبر استخدام الأنتيجين البرازى لميكروب الهليكوباكتر وسيلة دقيقة وبسيطة لتشخيص العدوى ويمكن استخدامها فى مرضى عسر الهضم للحالات التي لا تستدعي الفحص بمنظار المرىء والمعدة. وجود ارتفاع ملحوظ فى التعبير عن السيكلوأكسيجينيز-٢ وكذلك الانترليوكين-٨ فى أنسجة الأغشية المخاطية المبطنة لجدار المعدة المصابة بعدوى الهليكوباكتر قد يكون مؤشرا لمدى تضرر الأنسجة وقابلية تطور الضررالى تكوين خلايا سرطانية مما قد يستوجب علاج عدوى الهليكوباكتر حال وجود أعراض عسر الهضم.