African Journal of Microbiology Research Vol. 4(23), pp. 2543-2548, 4 December, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Microscopic changes of the gastric mucosa in dyspeptic patients infected by *Helicobacter pylori*

Reda Awadallah Saleh[1], Mohamed A. Dkhil[2,3]* and Saleh Al-Quraishy[2]

[1]Department of Anatomy, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.
[2]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.
[3]Department of Zoology and Entomology, Faculty of Science, Helwan University, Helwan, Egypt.

Accepted 28 October, 2010

Forty patients complaining from dyspepsia were used in this study. Exclusion criteria were recent or past history of gastric neoplasm of gastric surgery, long term therapy with nonsteroidal anti-inflammatory drugs and previous treatment with antibiotics or bismuth salts. Serum samples obtained from each patient were tested for the presence of IgG and IgA antibodies against *Helicobacter pylori* using standard ELISA. Gastric mucosal biopsies specimens were obtained from each patient, by gastroscopy. Specimens were fixed for light and scanning electron microscopic preparations. Light microscopic examinations showed the inflammatory cellular infiltration. By scanning electron microscopy more than 50% of biopsies appeared with mucosal ulceration, atrophy and metaplasia. The study demonstrates the destructive effect of *H. pylori* on the gastric mucosa.

Key words: *Helicobacter pylori*, gastric mucosa, light and electron microscopy.

INTRODUCTION

A previous study demonstrated that an organism in the form of a double helix, previously unknown was present on the surface of the mucosa of the human small bowel biopsies obtained at enteroscopy (Potter et al., 1995). The organism which appeared to be between 5 and 30 µm long was invariably seen lying on, but never invading, the small bowel mucosa. It was thought to inhabit the true small bowel rather than the duodenum. Preliminary findings suggested that the organism was present in any individual in high numbers and although often found in patients suffering from rheumatic complaints, it was subsequently found in patients investigated for non ulcer dyspepsia (Davis et al., 1995; Salem et al., 2010).

It is uncertain that endoscopic changes are characteristic of gastritis in subjects infected with *Helicobacter pylori*, one study found that this disease could not be diagnosed from the endoscopic appearance alone (Khakoo et al., 1994; Khan et al., 1999; Al-Enezi et al., 2010). However, antral nodularity is a common and specific finding in pediatric patients with *H. pylori* infection (Amini-Ranjbar and Nakhaee, 2008).

Persistent infection with *H. pylori* is now accepted as being a major risk factor for the development of gastric adenocarcinoma (Correa and Houghton, 2007; Hussein, 2010). However, most *H. pylori* infected persons never develop these neoplasms (Taylor and Blaser, 1991) and there is a need to identify additional factors that may influence the risk for the development of gastric cancer among *H. pylori* infected patients. Some of these factors will likely be host-related, whereas others may be related to differences among the infecting bacterial strains. We know that *H. pylori* strains are highly diverse (Akopyanz et al., 1992; Fujimoto et al., 1994; Truong et al., 2009) and that approximately 60% of isolates possess a gene cytotoxin-associated gen A, (Cag A) which stimulate gastric mucosal cells to produce high levels of interlukin-8 and a cytokine that has a vital role in the inflammatory response to infection (Truong et al., 2009). In addition, some Cag loci are homologue to genes of other species involved in transport systems (Truong et al., 2009).

For these reasons *H. pylori* strain that express the Cag, A protein are considered to be endowed with increased pathogenicity. Serological and microbiological studies

*Corresponding author. E-mail: mohameddkhil@yahoo.com. Tel: 00966-14675754. Fax: 00966-14678514.

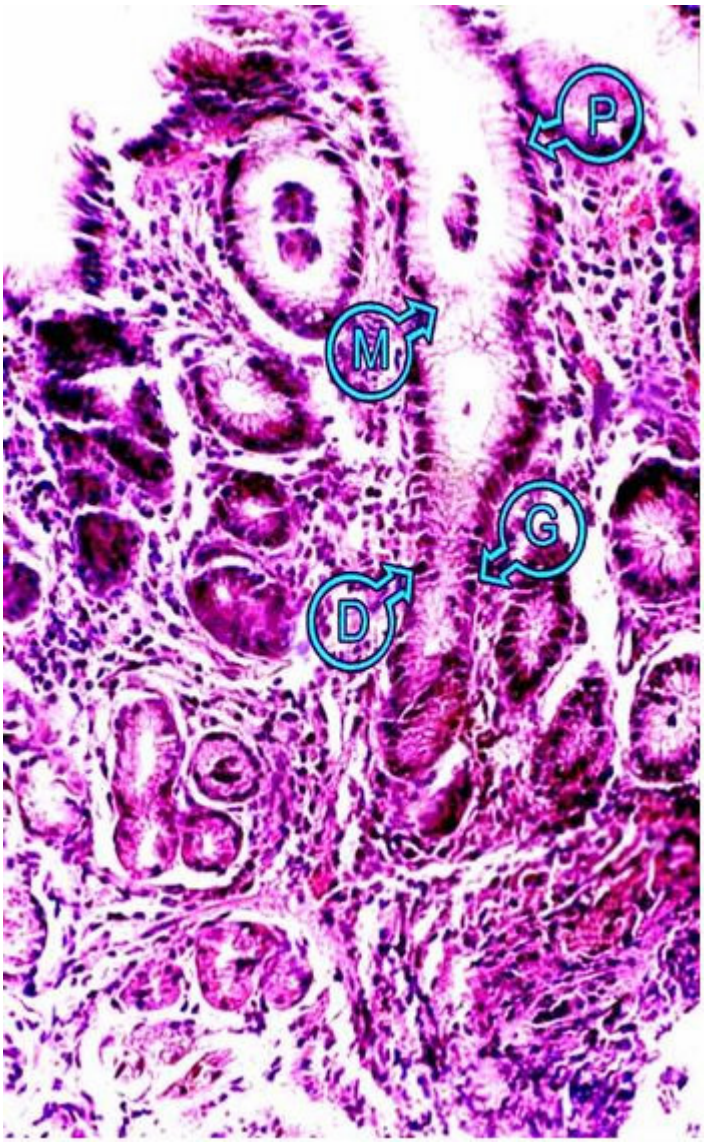

Figure 1. Photomicrograph of the mucosa of the pyloric antrum showing coiled and branching glands, which are lined by mucus cells (M), chief parital cells (D and G) and parital cells (p) (H and E, × 100).

have indicated that CagA. Positive strains are associated with enhance induction of a local inflammatory response (Oderda et al., 1993; Henriksnäs et al., 2009) and several studies have shown that the prevalence of antibodies to the CagA protein is higher in patients with peptic ulcer disease than in those with *H. pylori* gastritis without ulcer (Truong et al., 2009). Moreover, it has recently been reported that infection with Cag A-positive *H. pylori* strains may be associated with increased risk for development of gastric cancer (Blaser et al., 1995; Truong et al., 2009).

Here we identified mucosal changes associated with gastritis in patients infected with *H. pylori* using light and scanning electron microscopic studies in a case, control study, making comparisons with patients who were not infected with the bacterium.

MATERIALS AND METHODS

Forty dyspeptic patients who consecutively underwent upper gastrointestinal endoscopy from May 2008 - November 2009 were studied. Gastric biopsy specimens and serum samples were obtained from each patient. All subjects had given informed consent for study inclusion and the protocol had been approved by the local ethical committee in gastroenterology department, Al-Hussein University hospital, Cairo, Egypt. Exclusion criteria were a present or past history of gastric neoplasm or gastric surgery, long term therapy with nonsteroidal antiinflamatory drugs and previous treatment with antibiotics or bismuth salts. Endoscopy was performed after an overnight fast with standard upper gastrointestinal endoscopes and biopsy specimens were obtained with standard biopsy forceps. Five antral biopsies were taken for histological examination from the lesser curvature: two within 2 to 3 cm form the pylorus, one from the mid portion of the antral lesser curvature.

Serum samples, stored at -20°C, were tested for the presence of IgG and IgA antibodies against *H. pylori* using a standard ELISA (Chen et al., 2002).

Mucosal biopsies of the stomach were washed out by agitation in 5 ml of ice-cold glutaraldehyde 2% in 0.05 M phosphate buffer, pH 7.4 and further processed with 48 h (Malick and Wilson, 1975). They were dehydrated in a graded acetone series and critical point dried from liquid CO_2. Finally the specimens were sputter-coated lightly with gold and viewed in a JEOL JSM 6310 SEM. Other mucosal biopsies were fixed and further processed for paraffin section then stained with haematoxylein and eosin (H and E) for light microscopic investigation.

RESULTS

Light microscopic features of antral mucosa characterize by the presence of coiled and branching antral glands, which are lined by mucus cells that interspersed with endocrine cells (G and D types) and few partial cells (Figure 1). The glands in the mucosa of the body are straight tubes that constitute hydrochloric acid-producing partial cells, with scattered mucus cells in their upper portion and chief cells in their lower portion (Figure 2). Prevalence of inflammatory cells (Figure 3) are associated with infected specimens. Scanning electron microscopic studies revealing the features and cell types found in the antral and body type mucosa. Transitional type mucosa was found (Figure 4). Infected specimens are associated with an increased prevalence of antral ulceration, atropy (Figure 5) and metaplasia (Figure 6).

DISCUSSION

In the present study, it was found that the atrophic gastritis and metaplasia occurred predominantly at the gastric antrum is consistent with previous observation (El-Omar et al., 2005; Mach et al., 2007). It also found that the prevalence of metaplasia was significantly higher at

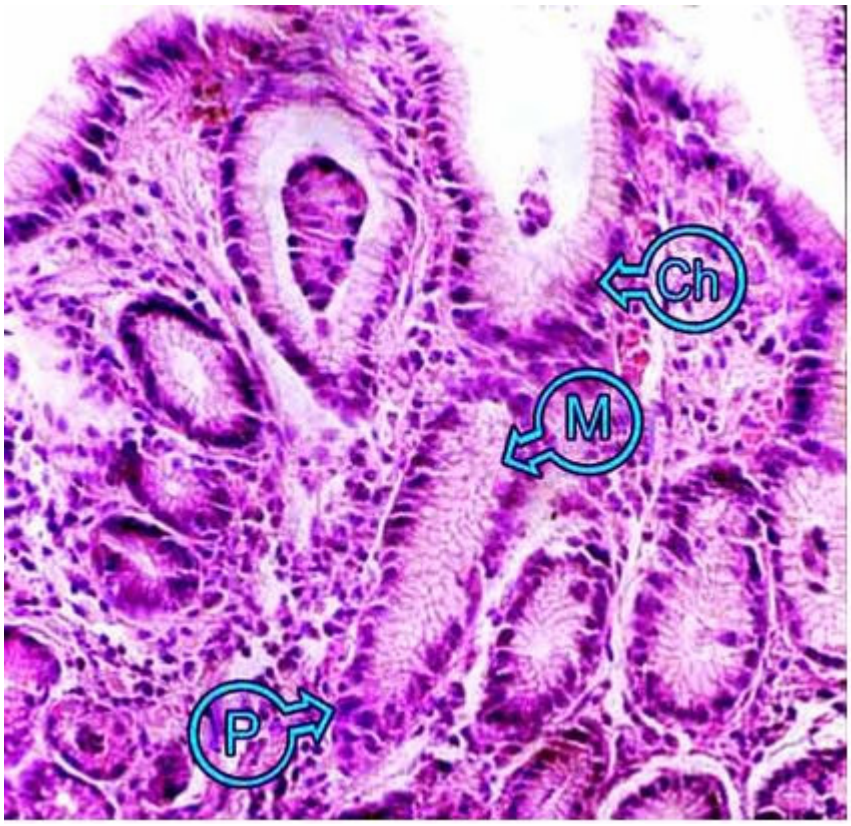

Figure 2. Photomicrograph of the mucosa of the body of the stomach, parital showing straight glands, lined by parital cells (p), Mucus cells (M) in their upper portion and chief cells (ch) in their lower portion (H and E, × 100).

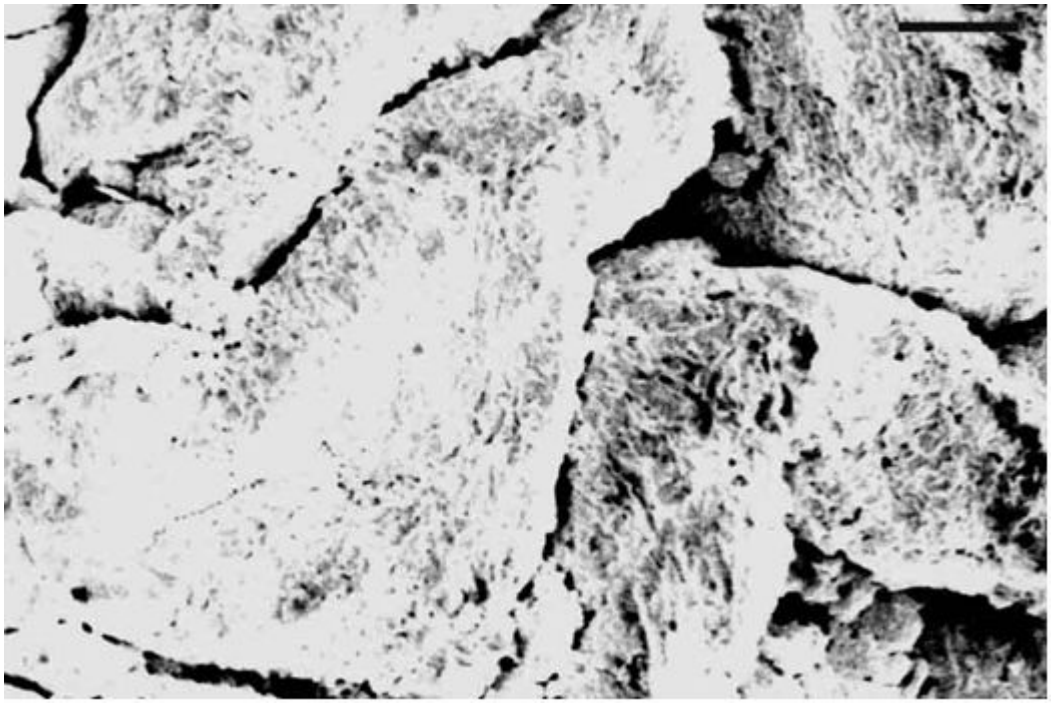

Figure 3. Scanning electron micrograph of the junction between body and pyloric antrum showing, transitional mucosa. Scale bar = 10 µm.

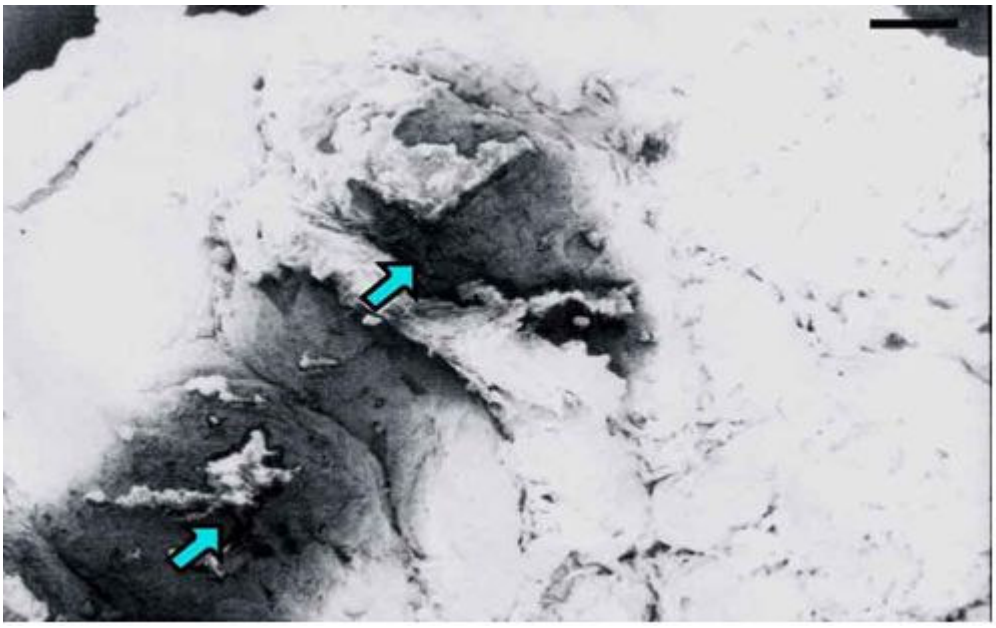

Figure 4. Scanning electron micrograph of the mucosa of pyloric antrum showing, mucosal ulceration and atrophy (arrow). Scale bar = 100 µm.

the gastric antrum of patients with *H. pylori* infection, compared with uninfected cases. Moreover, the prevalence of atrophic gastritis and metaplasia was significantly higher at the gastric incisura of infected patients than in those without infection. Studies before showed that gastritis first affected the distal part of stomach, tended to be more severe at the antrum and showed pylorcardial extension with greater extension to the gastric angulus in particular (Kimura, 1972; Alireza et al., 2008).

Development of gastric metaplasia is closely related to the acid-secreting function in the stomach, that is, the frequency and extent of metaplasia increases when the acid secreting area decreases (Tatsuta et al., 1979). Previous studies have suggested that gastric atrophy and metaplasia are more prevalent in gastric ulcer, which is believed to be associated with a low local acid secretion (Naomi et al., 2001; El-Omar et al., 2005; Alireza et al., 2008). These observations were confirmed in the present study. The development of gastric cancer in humans has been shown to be a multistep process, ranging from chronic gastritis to atrophy, intestinal metaplasia, dysplasia and finally invasive cancer (Ohkusa et al., 2000; Stolte et al., 2006). Preliminary pathological studies suggest that *H. pylori* play a role in initiating the sequential stages; that is causing chronic gastritis and atrophy (Coticchia et al., 2006; Gill et al., 2007). However, how and why *H. pylori* induced gastritis progress to intestinal metaplasia, a precancerous condition and probably the irreversible point , is yet be clarified. It is conceivable that *H. pyroli* infection induces the expansion or migration of antral mucosa by initiating a cycle of damage and regeneration of the gastric epithelial cells, and precipitates the process of intestinal metaplasia by increasing proliferation of the gastric epithelial cells (Kreuning et al., 1994). Numerous researchers found correlation between serum IgG level and the density of *H. pylori* colonization o gastric mucosa.

However, the results of the concentration of antibodies in IgG class should be considered critically due to their individual high changeability over time. Nevertheless, their very high level may prove that inflammatory/ immunologic changes in gastric mucosa are responsible for epigastric pain. The reports of other authors are also worth mentioning and they indicate that in subjects with gastric cancer the concentration of anti-*H. pylori* antibodies are lower than in superficial and atrophic gastritis (Manoj lovic et al., 2008). In conclusion, We should investigate dyspeptic patients firstly for the presence of antibodies against *H. pylori*. In positive cases, in addition to medical treatment endoscopic biopsy should be taken for histological examination. Infection with *H. pylo*ri initiating a cyclic damage and regeneration of gastric epithelium and precipitates the process of intestinal metaplasia by increasing proliferation of gastric epithelial cells.

ACKNOWLEDGMENT

This work was gratefully supported by the centre of Excellence for Biodiversity Research, College of Science, King Saud University, Riyadh, Saudi Arabia.

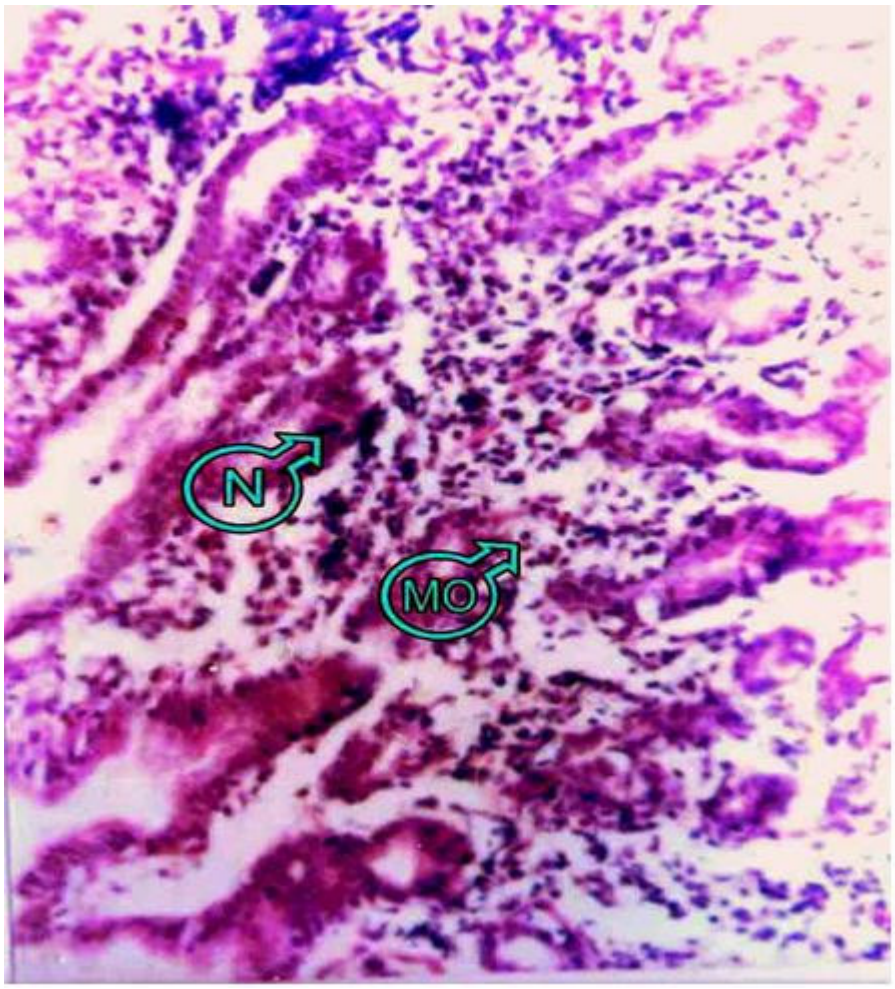

Figure 5. Photomicrograph of the mucosa of pyloric antrum showing mucosal infiltration by inflammatory cells, neutrophil (N) and mononuclear cells (Mo) (H and E, × 100).

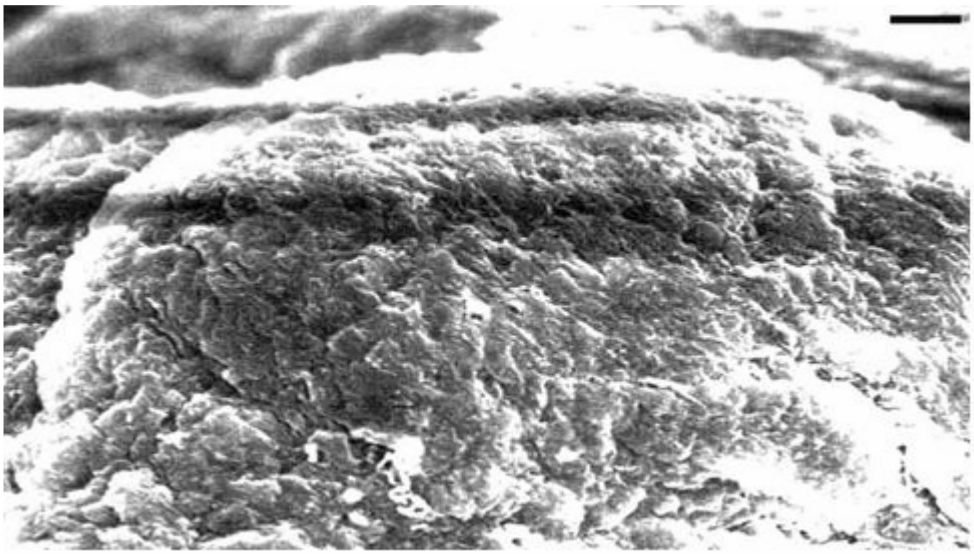

Figure 6. Scanning electron micrograph of the mucosa of pyloric antrum showing mucosal metaplasia. Scale bar = 10 µm.

REFERENCES

Akopyanz N, Bukanov NO, Westblom TU, Kresovich S, Berg DE (1992). DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD finger printing. Nucleic Acid Res., 20: 5137-5142.

Alireza A, Farid A, Hossein F, Firouzeh M (2008). Histological change in gastric biopsies from *Helicobacter pylori*-infected patient with and without peptic ulcer. Iranian J. Pathol., 3: 35-39.

Al-Enezi SA, Alsurayei SA, Aly NY, Ismail AE, Ismail WA, Al-Brahim N, El-Dousari A (2010). Endoscopic nodular gastritis in dyspeptic adults: prevalence and association with Helicobacter pylori infection. Med. Princ. Pract., 19: 40-45.

Amini-Ranjbar S, Nakhaee N (2008). Diagnostic Utility of Nodular Gastritis in Children with Chronic Abdominal Pain Undergoing Endoscopy. Am. J. Agric. Biol. Sci., 3: 494-496.

Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A (1995). Infection with *Helicobacter pylori* strains possessing cag A is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res., 55: 2111-2115.

Chen TS, Li FY, Chang FY, Lee SD (2002). Immunoglobulin G antibody against *Helicobacter pylori*: clinical implications of levels found in serum. Clin. Diagn. Lab. Immunol., 9: 1044-1148.

Correa P, Houghton J (2007). Carcinogenesis of *Helicobacter pylori*. Gastroenterol., 133: 659-672.

Coticchia JM, Sugawa C, Tran VR, Gurrola J, Kowalski E, Carron MA (2006). Presence and density of *Hclicobacter pylori biofilms* in human gastric mucosa in patient with peptic ulcer disease. J. Gastrointest. Surg., 10: 883-889.

Davies GR, Benson MJ, Gertner DJ, Van someren RMN, Rampton DS, Swain CP (1995). Diagnostic and therapeutic push type enteroscopy in clinical use. Gut., 37: 346-352.

El-Omar EM, Oien K, Murray LS, El-Nujumi A, Wirz A, Gillen D, Williams C, Fullarton G, McColl KE (2005). Increased prevalence of precancerous changes in relatives of gastric cancer patients. Gastroenterol., 118: 22-30.

Fujimoto S, Marshal B, Blaser MJ (1994). PCR-based restriction fragment length polymorphism typing of *Helicobacter pylori*. J. Clin. Microbiol., 32: 331-334.

Gill P, Amini M, Ghaemi A, Shokouhizadeh L, Abdul-Tehrani H, Karami A, Gilak A (2007). Detection of *Helicobacter pylori* by enzyme-linked immunosorbent assay of thermophilic helicase-dependent isothermal DNA amplification. Diagn. Microbiol. Infect. Dis., 59: 243-249.

Henriksnäs J, Atuma C, Phillipson M, Sandler S, Engstrand L, Holm L (2009). Acute effects of *Helicobacter pylori* extracts on gastric mucosal blood flow in the mouse. World J. Gastroenterol., 15: 219-225.

Hussein NR (2010). Helicobacter pylori and gastric cancer in the Middle East: a new enigma?. World J. Gastroenterol., 16: 3226-3234.

Khakoo SI, Lobo AJ, Shepherd NA, Wilkinson SP (1994). Histological assessment of the Sydney classification of endoscopic gastritis. Gut., 35: 1172-1175.

Khan MQ, Alhomsi Z, Al-Momen S, Ahmad M. (1999). Endoscopic features of *Helicobacter pylori* induced gastritis. Saudi J. Gastroenterol., 5: 9-14.

Kimura K (1972). Chronological transition of the fundic pyloric border determined by stepwise biopsy of the lesser and greater curvatures of the stomach. Gastroenterol., 63: 584-592.

Kreuning J, Lindeman J, Biemond I, Lamers CB (1994). Relation between IgG and IgA antibody titres against *Helicobacter pylori in* serum and severity of gastritis in asymptomatic subjects. J. Clin. Pathol., 47: 227-231.

Mach T, Skwara P, Biesiada G, Cieśla A, Macura A (2007). Morphological changes of the upper gastrointestinal tract mucosa and *Helicobacter pylori* infection in HIV-positive patients with severe immunodeficiency and symptoms of dyspepsia. Med. Sci. Monit., 13: CR14-9.

Malick LE, Wilson RB (1975). Modified thiocarbohydrazide procedure for scanning electron microscopy: routine use for normal, pathological, or experimental tissues. Stain Technol., 50: 265-269.

Manojlovic N, Babic D, Filipovic-Ljeshovic I, Pilcevic D (2008). Anti *Helicobacter pylori* IgG and IgA response in patients with gastric cancer and chronic gastritis. Hepatogastroenterol., 55: 807-813.

Naomi U, Shiro O, Soichioroy Y, Soichiro Y, Nobutoshi M, Shuji Y, Michio Y, Kiyomi T, Naomi S, Ronald J S (2001). *Helicobacter pylori* infection and development of gastric cancer. New Engl. J. Med., 11: 784-789.

Oderda G, Figura N, Bayeli PF, Basagni C, Bugnoli M, Armellini D, Altare F, Ansaldi N (1993). Serologic IgG recognition of *Helicobacter pylori* cytotoxin-associated protein, peptic ulcer and gasrodudenal pathology in childhood. Eur. J. Gastroenterol. Hepatol., 5: 695-699.

Ohkusa T, Fujiki K, Takashimizu I, Kumagai J, Tanizawa T, Eishi Y (2000). Endoscopic and histological comparison of nonulcer dyspepsia with and without *Helicobacter pylori* infection evaluated by the modified Sydney system. Am. J. Gastroenterol., 95: 2195-2199.

Potter U, Collins AJ, Notarianni LJ, Morris AJ, Smith AW (1995). Spirochaetes in small bowel. Lancet., 18: 345-723.

Salem EM, Yar T, Bamosa AO, Al-Quorain A, Yasawy MI, Alsulaiman RM, Randhawa MA (2010). Comparative study of Nigella Sativa and triple therapy in eradication of Helicobacter Pylori in patients with non-ulcer dyspepsia.Saudi J. Gastroenterol., 16: 207-214.

Tatsuta M, Okuda S, Taniguchi H, Tamura H (1979). Relation of intestinal metaplasia to the acid-secreting area. Endoscopy, 11: 166-171.

Truong BX, Mai VT, Tanaka H, Ly le T, Thong TM, Hai HH, Van Long D, Furumatsu K, Yoshida M, Kutsumi H, Azuma T (2009). Diverse characteristics of the CagA gene of *Helicobacter pylori* strains collected from patients from southern Vietnam with gastric cancer and peptic ulcer. J. Clin. Microbiol., 47: 4021-4028.

Taylor DN, Blaser MJ (1991). The epidemiology of *Helicobacter pylori* infection. Epidemiol. Rev., 13: 42-59.