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Possible Correlation between Helicobacter pylori and Duodenitis in Non-ulcer Dyspeptic Iraqi Patients.

إمكانية الترابط بين بكتريا *Helicobacter pylori* والتهاب الاثنا عشري في مرضى عسر الهضم غير المصاحب لقرحة في العراق

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Abstract :

In this study, we have attempted to show the relations between degrees of duodenal mucosal inflammation in biopsy specimens from non-ulcer dyspeptic (NUD) patients with the presence of anti–*Helicobacter pylori* (*H.pylori*) IgG. For this purpose, duodenal biopsies, taken during orogastroduodenoscopy, and sera were obtained from 42 consecutive NUD patients. Anti –*H.pylor*i IgG levels in those patients were determined using ELISA and correlated with histopathological findings. Five cases (11.9%) have demonstrated normal mucosa without any sign of inflammation. Mild inflammation was shown in 26 patients (61.9%) while sever inflammation was shown in 11 cases (26.2%). A positive correlation was found between presence of anti- *H.pylori* IgG and degree of inflammation of the duodenal mucosa (r = 0.306, p= 0.049). However, no correlations could be found with either the histological changes of the duodenal villi and endocopical findings. These results may indicate a causal relationship between *H.pylori* and duodenitis in nonulcer dyspeptic patients and, moreover, may implicate duodenitis as an important etiological cause of NUD.

الخلاصة :

لقد هدفت هذه الدراسة إلى محاولة إظهار العلاقة بين درجات التهاب الطبقة المخاطية للاثنا عشري مع تواجد الأجسام المضادة لبكتريا *Helicobacter pylori* في نماذج خزع ومصل أخذت من مرضى يعانون عسر هضم غير المصاحب بقرحة. اخذت خزع من الاثنا عشري خلال عملية التنظير الداخلي و مصول دم من 42 مريض تظهر عليهم اعراض عسر هضم غير مصاحب لقرحة. حددت كمية الأجسام المضادة لبكتريا *H. pylori* باستعمال تقنية مقايسة الممتز المناعي المرتبط بالأنزيم (ELISA) و قورنت نتائج قياس الاجسام المضادة مع نتائج فحص الهستوباثولوجيا (الفحص النسيجي المرضي). كانت الطبقة المخاطية للاثنا عشري طبيعية وبدون أي علامة التهاب في خمس حالات (11.9%) في حين وجد التهاب الطبقة المخاطية للاثنا عشري البسيط في 26 مريض(61.9%). أما التهاب الطبقة المخاطية للاثنا عشري الحاد فقد وجد في 11 مريض (26.2%). ولقد وجد ارتباط موجب وذي مغزى احصائي بين وجود الأجسام المضادة لبكتريا *Helicobacter pylori* مع درجة وخامة التهاب الطبقة المخاطية للاثنا عشري.(r = 0.306, p= 0.049). في حين لم يكن هناك ارتباط مع التغيرات النسيجية في زغابات الاثنا عشري أو فحص التنظير الداخلي.ربما تدل هذه النتائج على احتمال وجود علاقة سببية بين بكتريا *Helicobacter pylori* والتهاب الاثنا عشري في المرضى الذين يعانون عسر الهضم غير المصاحب لقرحة كما تشير النتائج الى احتمالية كون التهاب الاثنا عشري احد العوامل المسببة والمهمة في نشوء عسر الهضم غير المصاحب لقرحة.

Introduction :

Dyspepsia is a term used for a group of upper abdominal or epigastric symptoms, such as pain, bloating, nausea, early satiety, heartburn or simply indigestion [1]. Most patients with dyspepsia do not have ulcers or cancers. In fact, the majority does not have a structural or biochemical explanation for their symptoms. Such patients are diagnosed as having nonulcer dyspepsia (NUD). Multiple potential pathogeneses have postulated for NUD [2]. Of note, duodenitis is a common finding in patients with non-ulcer dyspepsia[3]. Since the seventieths of the last century, duodenitis has been described as a separate endoscopic entity from duodenal ulcer [4,5,6]. However, macroscopic abnormalities of the duodenal mucosa reflect histologically active inflammation in only a proportion of patients and

histological changes may be present in endoscopically normal mucosa[7]. Cluster and discriminant analysis indicated that the histological changes of the duodenum could be grouped by their statistical association into three simple categories: (a) normal, which includes many cases incorrectly labelled in some classification systems as mild or chronic duodenitis; (b) histologically defined mild duodenitis, characterized by an appreciable plasma cell response and oedema usually with intraepithelial polymorph infiltration and gastric metaplasia; and (c) severe duodenitis, with an appreciable polymorph response and villous atrophy but decreased plasma cells [8, 9]. H. pylori is a spiral shaped gram negative bacilli[10]. It is one of the most chronic infections associated with majority of cases of gastritis, 90% of duodenal ulcers and 75% of gastric ulcer[10]. Studies have shown high prevalence of H. pylori in patients of dyspepsia who have gastric mucosal abnormalities [11]. However, there is considerable controversy over whether or not a causal relationship exists between *H. pylori* infection and NUD. Recent work has found a strong relationship between NUD and *H. pylori* infection [12]. However, several large studies have failed to establish a causative relationship between *H. pylori* and NUD [13]. In an attempt to investigate the role of duodenitis in NUD and the search for an organic cause of duodenitis in our population, this study was undertaken to investigate the relationship of duodenitis and *H.pylori* infection NUD in Iraqi patients.

Materials and Methods :

This study was conducted in Al-Kadhmiya Teaching Hospital/ Baghdad during the period from January to December 2007. Patients attending the Endoscopy Unit above the age of 15 years, with dyspepsia of more than one month duration were included in the study irrespective of sex, ethnic group and socioeconomic status. Accordingly, multiple biopsies from 42 patients selected from patients who underwent routine upper gastrointestinal endoscopy for a variety of reasons including epigastric pain or discomfort, heartburn etc. were taken from mucosa duodenal bulb/or first or second part of duodenum. Patients with duodenal ulcers or have celiac disease were excluded from this study. No attempt was made to match the morphologic changes with the clinical symptoms. From each of 42 patients, sera were collected at the same time of the endoscopy. Biopsies were obtained through endoscopic biopsy forceps directly at the endoscopically abnormal mucosa in each area or randomly at the site if the mucosa appeared normal. Biopsies were immediately fixed in 10% formaldehyde, dehydrated in a graded series of ethyl alcohol solution, embedded in paraffin and cut into 3-5 μm thick sections. These specimens were stained with hematoxylin-eosin and then examined with light microscopy. Histological evaluations were performed by several expert pathologists in Al-Kadhmiya Teaching Hospital. According to the extent of lesions, duodenitis was divided into normal (as control group) {figure 1}, mild {figure 2}, moderate and severe degrees {figure 3} respectively referring to the Whitehead's classification and literature[3, 14,15,16,17,18]. For the detection of anti-*H.pylori* IgG antibodies an enzyme immunoassay technique (ELISA) was used (Biohit Plc. FIN-00880 Helsinki, Finland). This assay is based on the use of partially purified *H. pylori* bacterial antigen adsorbed on a microplate and a detection antibody labeled with horseradish peroxidase (HRP). The assay procedure, briefly, is in the following sequence: partially purified *H.pylori* bacterial antigen attached to the polystyrene surface of the wells binds the *H.pylori* IgG antibodies present in the sample; wells are washed to remove residual sample; an HRP-conjugated monoclonal anti-human IgG binds to the *H.pylori* antibodies; the wells are washed and the TMB-substrate is added (the substrate is oxy-genized by the enzyme and a blue colored end product is produced); and finally, the enzyme reaction is terminated with the stop solution and the *H.pylori* positive samples turn yellow with calculated values >38 EIU(enzyme immuno units) as recommended by the ELISA kit manufacturer. ***Statistical analysis.*** Data was analyzed using Statistical Package for Social Sciences (SPSS) version 15.0. A P value of <0.05 was considered to be statistically significant.

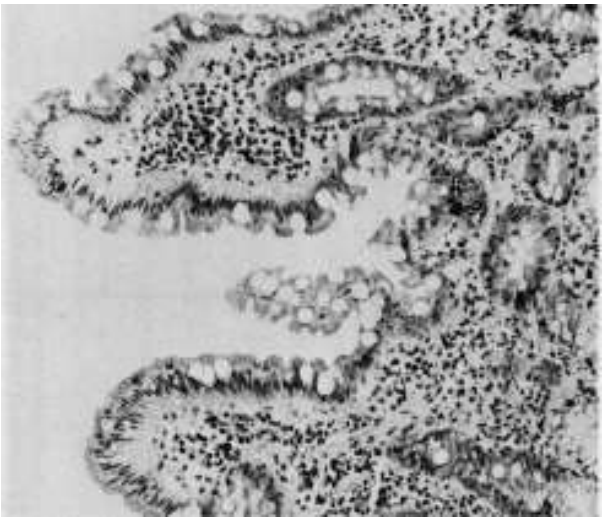

Fig. 1. Photomicrograph for representative example of normal defined statistically includes a range of villous morphology and plasma cell counts, but polymorphs are sparse. Original magnification x 125.

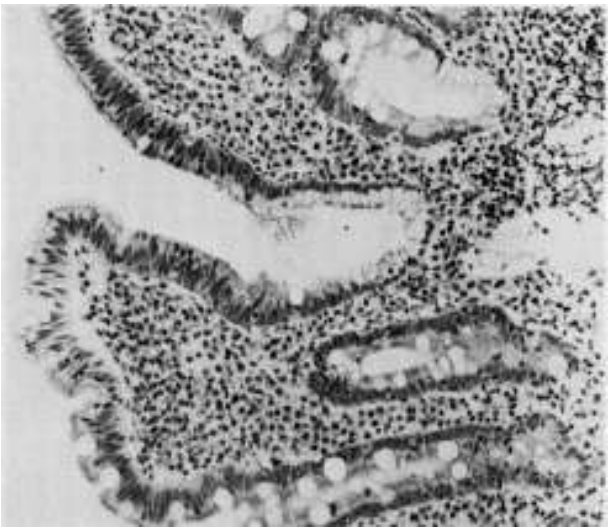

Fig. 2 Photomicrograph of biopsy Histologically defined mild duodenitis is characterised by increase in mucosal volume and plasma cell counts. Usually, there is also definite intraepithelial polymorph infiltration. Presence of polymorphs in crypt epithelium is a useful feature. Original magnification x 125.

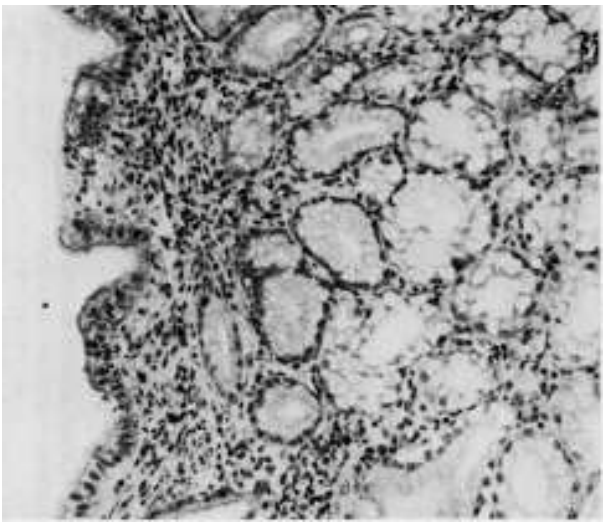

Fig. 3 Photomicrograph of severe duodenitis showing marked villous atrophy. Plasma cells are decreased but total cellularity is high as polymorphs are present in large numbers. Original magnification x 125.

Results :

Table 1 shows the patient's characteristics; mean age was 33.1±13.6 years with 20 (47.6%) males and 22 (52.4%) females. The endoscopic examination revealed that duodenitis was evident in only 11 cases while the rest (31 cases) showed non significant findings. The histological changes of mucosal biopsies from duodenum showed various patterns and, accordingly, the duodenal histological findings were reported as below. Five patients were found to have normal duodenal histology - with villus to crypt ratio greater than 2:1, in the presence or absence of Brunner's glands above the muscularis mucosa, but without any increase in the numbers of inflammatory cells. Mild chronic duodenitis was recorded in 26 pateints- characterized by the presence of intraepithelial mononuclear cell infiltration, an appreciable plasma cell response and oedema. In 11 patients, moderate to severe duodenitis was diagnosed based on the presence of an appreciable polymorph response and villous atrophy but decreased plasma cells. According to the examination of the duodenal villous architecture, 25 patients were shown to have intact villi with appearance of finger-like projections, while 17 patients were found to have villus changes. These villus changes ranged from mild broadening of the villi to villus atrophy.

Table (1) characteristics of patients.

Sex	Male	20 (47.6%)
	Fmale	22 (52.4%)
Age [minimum –maximum] (mean ± SD)	[15-65 year] (33.1±13.6)	
Endoscopic finding	No significant finding	31 (73.8%)
	Duodenitis	11 (26.2%)
Histopathology • Inflammation	Normal	5 (11.9%)
	Mild	26 (61.9)
	Severe	11 (26.2%)
Histopathology • Villar changes	Intact villi	25 (59.5%)
	Changes in villi	17 (40.5%)
Anti-H. pylori IgG antibody	Positive	22 (52.4%)
	Negative	20 (47.6%)

For the detection of anti- *H.pylori* IgG antibodies, we followed the instruction of the kit manufacturer: A negative value is less than 38 EIU and indicates no *H.pylori* antibodies detected or antibodies are below detectable level: A positive value is greater than 38 EIU indicates *H.pylori* antibodies detected. According the above cutoff value, 22 (52.4%) cases were considered positive and 20 (47.6%) were negative. As shown in table (2), only 20% (1 out of 5) of the patients with histological normal mucosa were found to be positive for *H.pylori*. This percentage is increased in patients with mildly inflamed mucosa to reach 50 % (13 out of 26). The highest percentage of the *H.pylori* positive was found within the group of patients with severe inflamed mucosa (72.7%). This trend of increasing of anti- *H.pylori* antibody positivity along with increased degree of inflammation severity was statistically significant as measured using Pearson's Correlation Coefficient (r = 0.306, p= 0.049). Regarding the histological architecture of the villi, no difference in anti- *H.pylori* antibody positivity between the group showed intact villi and the group that showed alterations in villous architectures where the two groups demonstrated nearly equal percentages (60%, and 58.8%, respectively), table (2).

Table (2) distribution of the positive *H. pylori* IgG antibody with degree of duodenal inflammation and histological changes of duodenal mucosa.

		Anti-*H.pylori* IgG antibody		Total
		negative	positive	
Inflammation	Normal	4 (80%)	1(20%)	5
	Mild inflammation	13 (50%)	13 (50%)	26
	Sever inflammation	3 (27.3%)	8 (72.7%)	11
Total		20	22	42
Pearson Correlation (*r*)		0.306(*)		
Sig. (2-tailed) (*p*)		0.049		
Histopathology	Intact Villi	10 (40%)	15 (60%)	25
	Changes of Villi	7 (41.2%)	10 (58.8%)	17
Total		17	25	42
Pearson Correlation		-0.012		
Sig. (2-tailed)		0.941		

* Correlation is significant at the 0.05 level (2-tailed).

Discussion :

Nonulcer dyspepsia (NUD) is a common syndrome of persistent ulcer like symptoms in the absence of radiographic and endoscopic abnormalities. Multiple potential pathogeneses have been postulated for NUD. Similarly, many different therapies have been tried. This multitude of diagnostic and therapeutic options simply underscores the fact that the true cause of dyspepsia is not known [2]. As a consequence, the first step toward successful management of dyspepsia is the identification of its true cause. In this study, we tried to investigate the role of duodentitis as a cause of NUD and the role of *H. pylori* infection as a causative agent of duodentitis in NUD. In the present study, most of our patients were found to have duodentitis (37 out of 42), however, the severity of inflammation was variable with mild duodentitis being the most predominant (26 cases). This result may emphasize the duodentitis as a potential cause of NUD. The latter conclusion is in consistent with the current hypotheses for NUD

etiology [3]. Moreover, in our attempt to find out the role *H.pylori* as an important organic cause of duodenitis, and, consequently, of NUD, we used ELISA technique to detect anti *H.pylori* IgG antibodies in the sera of our patients. ELISA technique has proved to show similar sensitivity and specificity in detecting *H.pylori* infection in dyspeptic patients as compared to the biopsy-based tests[19, 20]. We found an increasing in anti-*H.pylori* IgG positivity along with the increasing severity of the duodenitis and this increase was statistically significant. This result clearly implicates *H.pylori* infection as an organic cause of duodenitis in NUD. On the other hand, our results regarding high prevalence of H pylori antibody positivity in severe duodenitis cases may explain previous findings of several researchers. Hasan et al (1983) [18] suggested that duodenal mucosa endoscopically defined as showing 'mild' duodenitis did not differ significantly from endoscopically normal mucosa with respect to inflammatory cell counts. They showed that significant differences were present between areas of endoscopically 'severe' duodenitis and endoscopically normal control mucosa. This same trend was obvious in the cases examined in our study. Moreover, Concerning prevalence of *H. pylori* in non-ulcer dyspeptic patients, our results were comparable with other recent study undertaken in Libya that reported prevalence of 77% [19]. It is worthy to mention here that it has been suggested that gastric metaplasia in the duodenum and *H pylori* associated gastritis might be synergistic in the pathogenesis of duodenitis, with the metaplastic gastric epithelium allowing *H pylori* to colonise the duodenal mucosa, where it produces an acute inflammatory response [21]. In the present study we excluded all cases showed gastric metaplasia in the duodenum aiming to focus on duodenitis as a separate entity in isolation from gastric pathology. Finally we conclude that duodenitis may be a potential etiological factor of NUD and *H.pylori* may be an important organic cause of this duodentis.

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