Histological changes in antrum associated with *Helicobacter pylori* infection.

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Abstract:

Out of 136 cases 85 cases undergo histopathological test and age groups (20-80)were included 65 gastritis (48/65 (73.8%) male, 17/65 (26.1%) female, 13cases (9/13(69.2%) male,4/13(30.7%) female) mild gastritis (rare neutrophils seen) ,30 cases 24/30(80%) male,6/30(20%) female, moderate gastritis (obvious neutrophils within glandular and foveolarepithilum),22 cases (15/22(68.1%) male, 7/22(31.8%) female) severe gastritis (numerous neutrophils with glandular micro abscesses and mucosal erosion),12(8/12(66.6%) male,4/12(33.3%) female)ulcer and 8 normal (4/8 (50%) male, 4/8(50%) female).The statistical study refer that non significant differences between age groups and sexes .

Introduction

***Helicobacter pylori* (*H. pylori*)** is the main environmental factor contributing to the development of chronic gastritis and is associated with an enhanced risk of developing peptic ulcer and gastric cancer (Sipponen P,1993 ; Parsonnet J and Forman D, 2004).*H.pylori*has largely changed the understanding of the etiology of gastritis (Warren JR and Marshall B,1983) and atrophic gastritis has been shown to be a consequence of long term gastritis caused by *H. pylori* (Kuipers EJ*et al*.,1995),However, gastric auto antibodies have also been demonstrated in subjectswith *H pylori* positive gastritis(Clayes D *et al*., 1999) and a question has arisen of whether or not *H pylori* also plays a role in the etiology of autoimmune typeatrophic gastritis(Kuipers EJ *et al*.,1997).

Gastritis commonly refers to inflammation of the lining of the stomach**,** but the term is often used to encompass a variety of symptoms resulting from stomach lining inflammation, as well as symptoms of burning or discomfort(Sipponen,2007). True gastritis comes in several forms and is diagnosed using a combination of tests, in the 1990s scientists discovered that the main cause of true gastritis is infection from a bacterium called *Helicobacter pylori*(Dixon *et al*.,1996). All individuals infected by *H. pylori* establish gastritis. First , an acute inflammation occurs, which later turns into a less symptomatic variant of chronic superficial gastritis . Host immune mediators keep the infection under control ,but are still unable to clear the invader from the host. This results in persistent infection for life ,unless treated with antibiotics .Usually the infection is asymptomatic and the persons infected are not harmed by *H.*

pylori carriage .However for the 10-20% that develop disease, the consequences are serious and sometimes fatal(Suerbaum and P. Michetti,2002)

Acute gastritis: Infection with *H pylori* leads to an inflammatory response in the gastric mucosa (Malaty*et al*.,1999). In the acute phase, the infection leads to dense infiltration of polymorph nuclear leucocytes in to the gastric mucosa with formation of micro abscesses and exudation of inflammatory cells to the mucosal surface(Marshall *et al*.,1985). The whole stomach is affected in the initial phase leading to an almost total loss of acid secretion for up 40 days followed by normalization within 2-3months(Malaty*et al*.,1999). Acute infection may lead to unspecific symptoms such as dyspepsia, nausea, diarrhea for 1-2 weaks(Marshall*etal*.,1985).After this acute phase, the inflammation develops in to chronic active gastritis in the antrum(Meining*et al*., 2001)

Chronic gastritis: The most common cause for chronic gastritis is*H. pylori* infection (Meining*et al*., 2001). After the acute phase, the amount of lymphocytes in the mucosa increases as assign of chronic inflammation . The presence of both polymorph nuclear cells and lymphocytes in the mucosa characterizes chronic active gastritis where the former indicates the activity and the latter the chronic part of the inflammatory response (Kuipers*at al*.,1995). Lymphoid follicles may be present(Meining*et al*.,2001). In some individuals, amore intense antral inflammation is seen leading to increased gastrin release and subsequent increased acid secretion (El-Omer *et al*.,2000). This leads to increased

acid load and gastric metaplasia in the proximal duodenum, which can also be colonized by *H pylori*, and in some this can case duodenal ulceration(Meining*et al*.,2001). This phenotype has avery low risk for developing gastric cancer(Sipponen,2001).

Ulcer: Is defined as an disproportion of antagonistic aspects such as acid or pepsin and defensive factors such as mucus, bicarbonate and blood flow. This balance may be infected by *H. pylori*, as the bacterium is discovered in nearly 94% of duodenal ulcer and 84%of gastric ulcer cases (Kuipers*et al*.,1995).

Aim of study: This study aims to relationship between *H. pylori* infection and severe , moderate , mild gastritis and ulcer in antrum.

Materials and Methods:

The histological sections were prepared according to (Luna, 1960) as follows:

1- Fixation

Tissues were fixed for 24 hr. with 10% formalin freshly prepared by dissolving 100 ml from 10% formalin in a buffered solution(4g NaH2PO4 , 6.5g Na2HPO4 , 900 ml D.W).

2- Washing

Specimens were washed three times with distilled water .

3- Dehydration

Specimens were dehydrated through 50% , 70% , 90% , and 100% absolute ethanol alcohol for 2 hr. to each concentration .

4- Clearing

After dehydration the tissues were treated with a mixture of ethanol alcohol :

Xylene in ratios (3:1 , 1:3 , 1:1) for 1 hr. for each concentration, then left in pure xylene for 3 hr.

5- Infiltration

Samples were impregnated with mixture of xylene : paraffin, in ratios (1:3, 1:1, 3:1) for 2 hr. to each concentration, then transported to pure paraffin wax for 24 hr.

6- Embedding

After infiltration, the tissues were put in plastic blocks, paraffin wax poured on it, then left to become solid at room temperature.

7- Sectioning

5 micron sections were cut using rotary microtome, put in 60 ^{o}c water bath , then transferred to clean , dry slides covered with Mayer´s Albumin , and left on 35^{o}c hot plate for 24 hr.

8-Staining

Sections were stained by haematoxylene-eosin stain according to (Drury,1967) as follows :

- Sections were put in pure xylene for 3-5 min to melt paraffin wax.
- To remove xylene**,** sections were passed through serial concentrations of ethanol, 100%, 90%, 70%, 50%, for 2-3 min to each concentration.
- Washed with distilled water.
- Stained with haematoxylene stain for 5 min, afterwaredwashed with distilled water.
- Stained with eosin stain for 1-2 min, then washed with distilled water.
- Sections were passed through serial concentrations of ethanol alcohol, 50%, 70%, 90%, 100%, for 1 min to each concentrations**.**
- Sections were put in xylene for 5 min.
- Plasterisizied with Canada Balsam, then covered with cover slides.

●Examined and photographed by compound light microscope type

Statistical analysis:

Statistical analysis is done by using the SPSS software version15, the Chi-Square is used to assess statistical significance.

Results and Discussion: According to histopatholo-gical study was detected three groups under study.

Table (1) were showed that 8/85 (9.4 %) cases were normal, while 65/85(76.5%) cases were gastritis, , 13/65 (20 %) mild gastritis (the number of inflamm-atory cells were few), 30/65 (46%) moderate gastritis(the number of inflammatory cells were high),22/65 (34%) sever gastritis(the number of inflamm-atory cells were more than high the moderate) , and 12/85 (14.1%) cases were ulcers. Fig (1,2,3,4) shows the histopath-ological exam. In our present study no statistically significant differences ($P > 0.05$) between sexes and age groups. In this study *H. pylori* found in all persons with gastritis this results agreement with (Dooley *et al*.,1989).In some cases, *H.pylori*not found in the tissue or limitation also arise at times because an inadequate number of biopsy specimens obtained or failure to obtain specimens from different areas of the stomach(El-Ziamaity HM and Graham DY,1999) or because of patchy characteristics of atrophic chan-ges in stomach mucosa (Rugge M.P.Correa and M. Dixon,2002; Moussa, A.B. *et al*.,2004) . The specificity to (H&E) to detected *H. pylori* was 66% (Fallone*et al*.,1996), This results that may be due to some of *H. pylori* don't appear with red color and difficult to differentiated with tissue specially if the number of bacteria in tissue was little (Magraud and Lehours,2007),this results agreement with (Menda-ll*et al*.,1993), because that may

be put the patient recently before the eradicated of histological biopsy to treatment with antibiotics proton pump inhibitor (PPI), that acts to decrease the number of bacteria in gastric mucosa, which making more difficult to identification (Santacroce*et al.,* 2007).

Chronic *H. pylori* associated gastritis was a risk factorfor development of gastric atrophy and intestinal metaplasia that are knownpremalignant lesions(Correa *et al*.,1975).Chronic gastritis was diagnosed when any lymphocytes, however few, were present in the superficial part of the lamina propria. The degree of chronic inflammatory infiltrate correlates closely with the extent and density of *H. pylori*colonization and generally more severe in the antrum (Bayerdorffer*et al*., 1992) *H. pylori* was observed more in the severe gastric atrophy mucosa than thosewith mild gastric atrophy or normal mucosa. It was believed that the gastric atrophy lead to arose in the intragastric pH, which was saidto be unfavorable to the survival of *H. pylori* because ofa high pH associated with these two lesions. The findingin this study in which *H. pylori* was found more in severe atrophy (Henry Wabinga,2005). *H. pylori* preferentially colonize the antrum, but they may infect any part of the stomach where it causes gastritis. When treated, the bacteria migrate from the antrum to the corpus, decreasing the activity of antral gastritis. Markedneutrophilic infiltrates appear in the mucous neck region and lamina propria in early acute gastritis, when severe, they aggregate in the pit lumens to form pit abscesses. Both the neutrophils and the *H. pylori* destroy the epithelium, causing the mucous neck cells to proliferate in an effort to replace

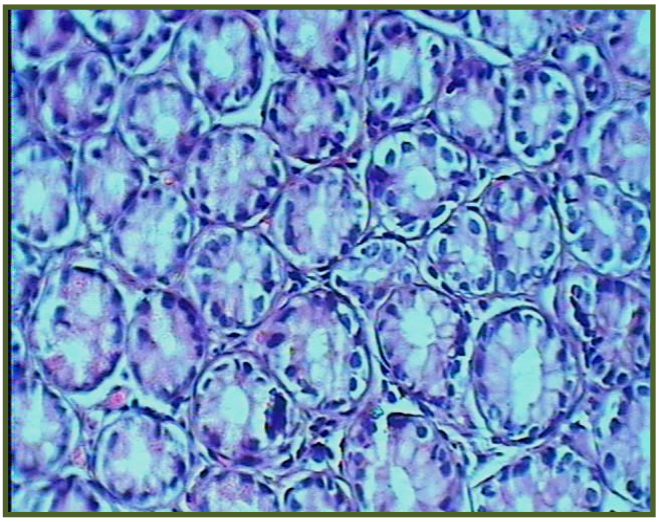
the dying cells (Genta*et al*., 1993). *H. pylori* in 22.1% cases of moderate chronic gastritis and 16.1% cases of severe chronic gastritis were *H. pylori* positive, whereas in mild gastritis 9.5% lower frequency of *H. pylori* was seen.

Lymphoid follicles are a common feature of *H. pylori* associated gastritis(Gonzalez and Agudo,2012 ; Delahay and Rugge, 2012).Lymphoid follicles may result from chronic antigenic stimulation in response to *H. pylori* (Correa and Piazuelo, 2012)

Table (1) Distribution of Histological examination according to age groups and sexes:

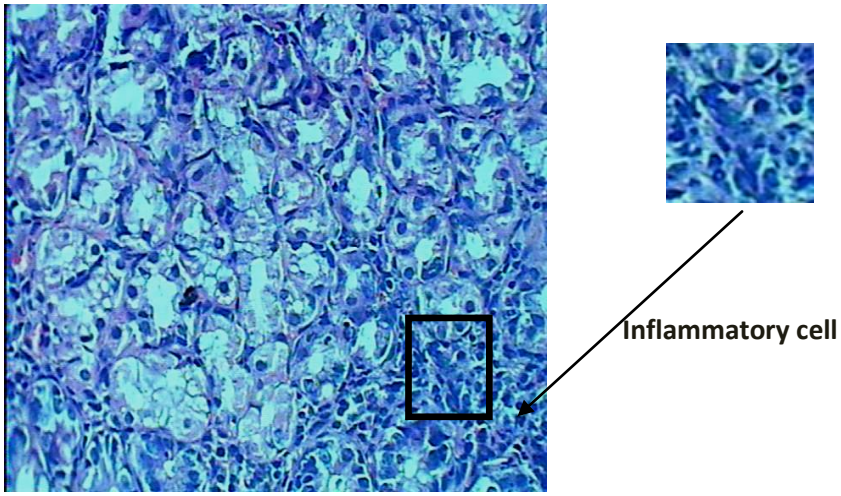
Age group / Sex	Gastritis											Total
	Mild		Moderate		Severe		Total	Ulcer		Normal		
	♂	♀	♂	♀	♂	♀		♂	♀	♂	♀	
20 - 30	**1**	**1**	**3**	**2**	**2**	**1**	**10**	**2**	**1**	**1**	**0**	**14**
30.1 - 40	**2**	**1**	**2**	**1**	**3**	**2**	**11**	**0**	**1**	**1**	**2**	**15**
40.1 - 50	**1**	**0**	**5**	**1**	**3**	**2**	**12**	**0**	**1**	**0**	**1**	**14**
50.1 - 60	**1**	**1**	**6**	**1**	**2**	**1**	**12**	**2**	**1**	**0**	**1**	**16**
60.1 - 70	**2**	**0**	**5**	**0**	**3**	**0**	**10**	**3**	**0**	**0**	**0**	**13**
70.1 - 80	**2**	**1**	**3**	**1**	**2**	**1**	**10**	**1**	**0**	**2**	**0**	**13**
Total	**13(20%)**		**30(46%)**		**22(**		**65(76.5)**	**12(14.**		**8(9.4)**		**85(100**

			34%)		**1)**		**%)**



Figure(1): Section of Stomach within normal limits

(H&E × 400), thikness 4-5µm



Figure(2) Section of stomach with mild gastritis associated with infiltration of inflammatory cells H&E×40,thickness(4-5)µm

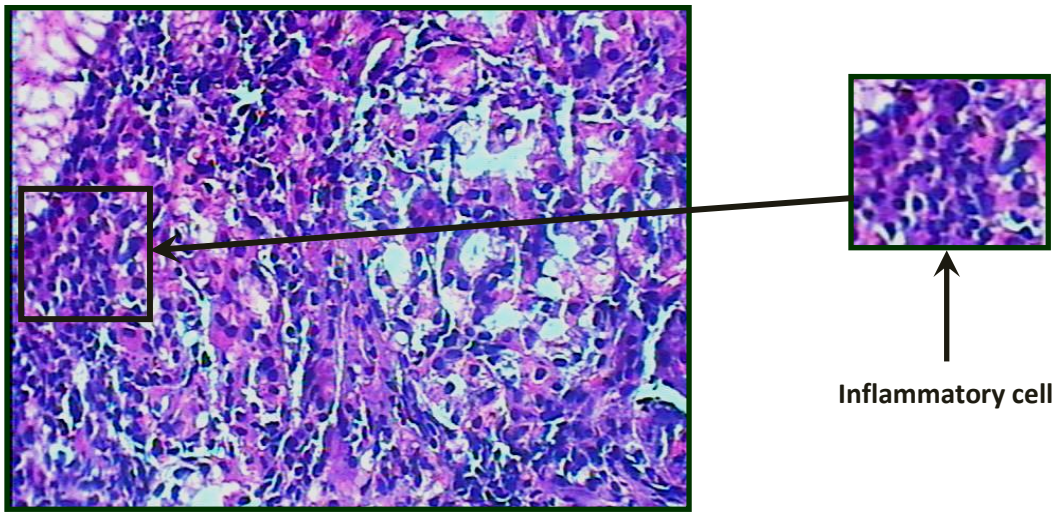


Figure (3) Section of stomach with moderate gastritis associated with infiltration of inflammatory cells H&E×400,thickness(4-5)µm

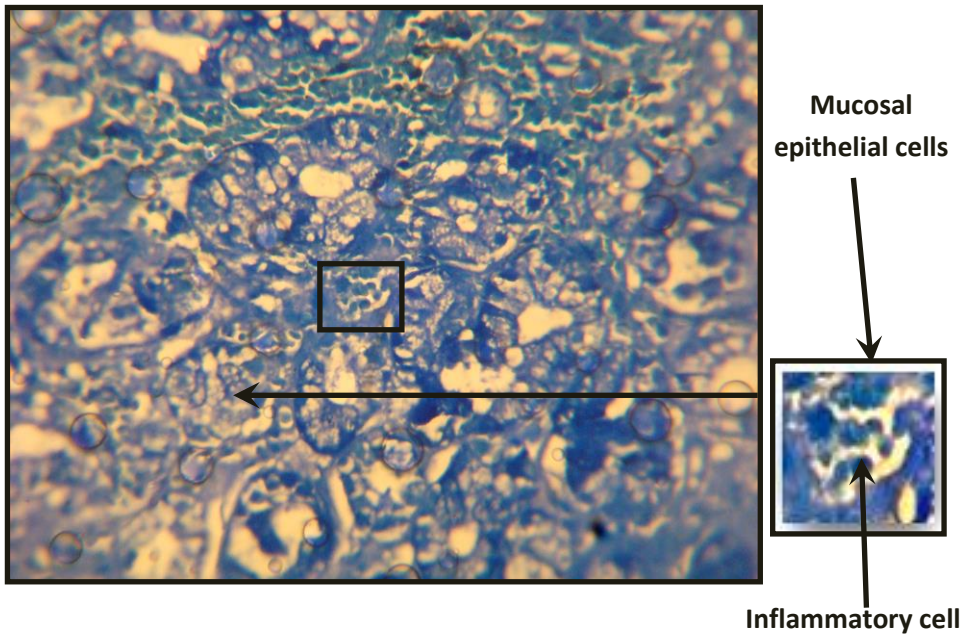


Fig (4) Section of stomach with severe gastritis associated with mixed infiltration of inflammatory cells (H&E×400) ,thickness(4-5)µm

Conclution: severity of gastritis and presence of *H.pylori* affect gastric epithelial cells(mild,moderate and severe gastritis) were limited acute infection of *H.pylori* .

Recommendations: The changes in the gastric mucosa such as mild , moderate or severe were differs from person to person ,however, suggested to studies other virulence factors of *H.pylori* strain.

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التغييرات النسيجية في البواب المرافقة للاصابات بجرثومة الملوية البوابية

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الخلاصة :

من مجموع 136 حالة مرضية خضعت للفحص النسيجي وكانت ضمن الفئات العمرية من (20-80) تضمنت 65 حالة التهاب المعدة منهم 48\65 (73.8%) ذكور و17\65 (26.1%) اناث، 13 حالة يعانون من التهاب المعدة الخفيف (نادرا ما تشاهد العدلات) منهم 9\13 (69.2%) ذكور و4\13 (30.7%) اناث. تعاني 30 حالة منهم من التهاب المعدة المتوسط (وضوح العدلات مع خلايا غدية طلائية) منهم 24\30(80%) ذكور و6\30 (20%) اناث .

22 حالة يعانون من التهاب المعدة الشديد (كثرة عدد الخلايا العدلة مع وجود تقيحات غدية وتاكل في النسيج، 15\22 (68.1%) ذكور و7\22 (31.8%) اناث وتعاني 12 حالة من القرحة ، 8\12 (66.6%) ذكور و4\12 (31.3 %) اناث. مع وجود 8 حالات طبيعية 4\8 .(50%) ذكور و4\8 (50%) اناث.اظهرتنتائج التحليل الاحصائي عدم وجود فرق معنوي بين المجاميع العمرية والجنس.