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Occurrence of*Helicobacter pylori* among Iraqi patients with suspected gastric ulcer:histopatological studyfor gastric mucosal biopsies

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ABSTRACT

Background :*Helicobater pylori* presents on the gastric mucosa of 20% of persons under 30 but the percent increase to more than 50% after age 60. For most people, it does not cause ulcers or any other symptoms but it might convert from acute to chronic infection and with time might cause undesired consequences.Objective : To investigate the occurrence of Helicobacter *pylori* among random Iraqi patients suspected with gastric ulcer or suffering from acute, mild and chronic gastritis and to determine the most accurate method for bacterial diagnosis.Methods: Samples were collected from different Baghdad hospitals\Iraq from September 2014 till January 2016.Two hindered and sixty random patients were undergoing screening test for the presence of*H.pylori*. Collected samples were serum to be used for *ECOtest* D-HP-32 (rapid detection device) and for ELISA serological test. Biopsies for the hisopathological study were obtained and stained either with E&H orGiemsa stains. Results: According to the rapid detection device *ECOtest*the prevalence of *H.pylori* was 102\260 (39.2%) and the percentage of infected male was 57\130(43.8%) which is higher than the female ratio when it was 45\130(34.6%). The higher infected aged group in male appeared between 41-50 followed by 40-31 years when the rates were 17\57(29.8%) and 12\57(21.1%), respectively .For women,the results showed that aged group 21-30 year and 31-40 were more predominant as compared with other groups and the rates were 18\45(40%) and 11\45(24.4%),respectively.According to ELISA 97\102 (--) were positive to *H.pylori*.Histopathological study for the endoscopic gastric biopsies revealed that most sections (stained with H&E) showed mucosal ulceration with either heavyacute, mild or chronic inflammatory cells infiltrate .Some mucosal ulceration was associated with mild chronic inflammatory cell infiltrates and mucosal glandular hyperplasia with congestion. It could be said that at least 10\35 (28.6%) of the examined stained sections showed chronic gastritis with heavy infiltrate cells while 6\35(17.1%) with mild gastritis.Conclusion: The *EOCtest* represent simple rapid and suitable method for primary checkup diagnosisespecially for random patients. Still Antibodies specific-IgG ELISA is more specific as it reflect the tendency of infection and titers of antibodies. Histopathlogical study for biopsies is reasonable for analysis active and chronic infections to prevent progressing of the disease

KEYWORDS:*Helicobacter pylori* ,*EOCtest* , Antibodies specific-IgG ELISA , Histopathological study.

INTRODUCTION

Helicobater pylori is a fastidious gram negativespiral to curved rod shaped.The bacteria presents on the gastric mucosa of 20%of persons under 30, 50% after age 60 years and the percentage might reached to 80% in adult of developing countries[1]. About two-thirds of the world's population has it in their bodies however, itdoes not cause ulcers or any other symptoms[2]. It considered one of the most common bacterial infections worldwide and a leading cause of dyspepsia and causative agent of type B active chronic gastritis[3],gastric

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lesions and some cases of duodenal ulcers[4]. *H.pylori*stimulatesboth of cellular andhumeral immune systemand specific antibodies will evoke against the bacteria[5].Almost 1% of infected patients might develop gastric adenocarcinoma, and vast majority of gastric cancers arise from infection with *CagA+ H. pylori* strains[6]. *H.pylori* can be identified either via serological test (ElISA), Rapid urease test,or directly from gastric biopsies beside the molecular detection using PCR technique[7,8]. Rapid detection is essential to prevent developing the state from simple gastric or duodenal ulcer to gastric cancer,since there are evidencesbetween*H.pylori,* gastric cancer and gastric lymphoma[2]. In this study three different methods were used to diagnose *H. pylori*in patients suspected to have gastric ulcer .this include using rapid detection device, serological test followed by hisopathological study

MATERIAL AND METHODS

Patients and Samples collection: Sampleswere collected from different hospitals during September 2014 till January 2016.Two hindered and sixtyvolunteers patients who suspected to have gastric ulcer were undergoing screening test for the presence of *H.pylori*.Those patients were sufferingfrom burningsensation, pain withuncomfortable feelingspecially empty stomach. A Blood sample were collected without anticoagulant, centrifuged at 20 C◦, 5000 rpm for 5 minutes to separate serum and was stored at -20 c◦ to be usedlater. Thirty-five biopsies were obtainedfrom inpatientsthatshowed positive result in the primary diagnostic test. During endoscopy, the specialist doctor passes a hollow tube equipped with a lens (endoscope), searching for ulcers in stomach lining layer.Biopsies were immediately fixedusing10% buffered formalin to be usedfor the histopathlogical study.

Rapid diagnosis test for H.pylori infection:

*H.pylori*Antibodies Rapid Test Device (serum \plasma) was used as a rapid visual immunoassay for the qualitative presumptive detection of specific IgM and I gG antibodies to *H.pylori* in human serum or plasma specimens .The procedure was done according to manufacture instructions (*ECOtest* D-HP-32). The device and the specimens were brought to room temperature and 75µl from the serum was transferred to specimen well. Migration of specimen across the result area in the center of the device will cause coloration (dark red color) of control band and another red band appeared within five minutes in case of positive result.Deep of the color and time of result appearance was recorded.

Quantitative determination of IgG-class antibodies againstH.pylori using Enzyme linked immunosorbentassay (ELISA):

The process was done according to manufacture instructions ofIgG-class antibodieskit (Novalisa*H.pylori*immune diagnostic GMbH Germany 2015). The process requires reaction between diluted serum samples and *H.pylori* anti-IgG Conjugate. The process involved repeated steps of washing and incubation time followed by Stop Solution .The absorbance of the specimen was performed at 450/620nm within 30 min after addition of the Stop Solution .Measurement adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.Positive results were compared with the negative control in line A 1 ,2 and the third well.

Hisopathlogicalstudy:

Paraffin embedded tissue blocks were prepared and5µm thickness sections were mounted on slides for Hematoxylin and Eosin staining. Mucosal ulceration with heavy acute or chronic inflammatory cells infiltrate were detected . Giemsa stain was used to search for bacteria within the tissue.

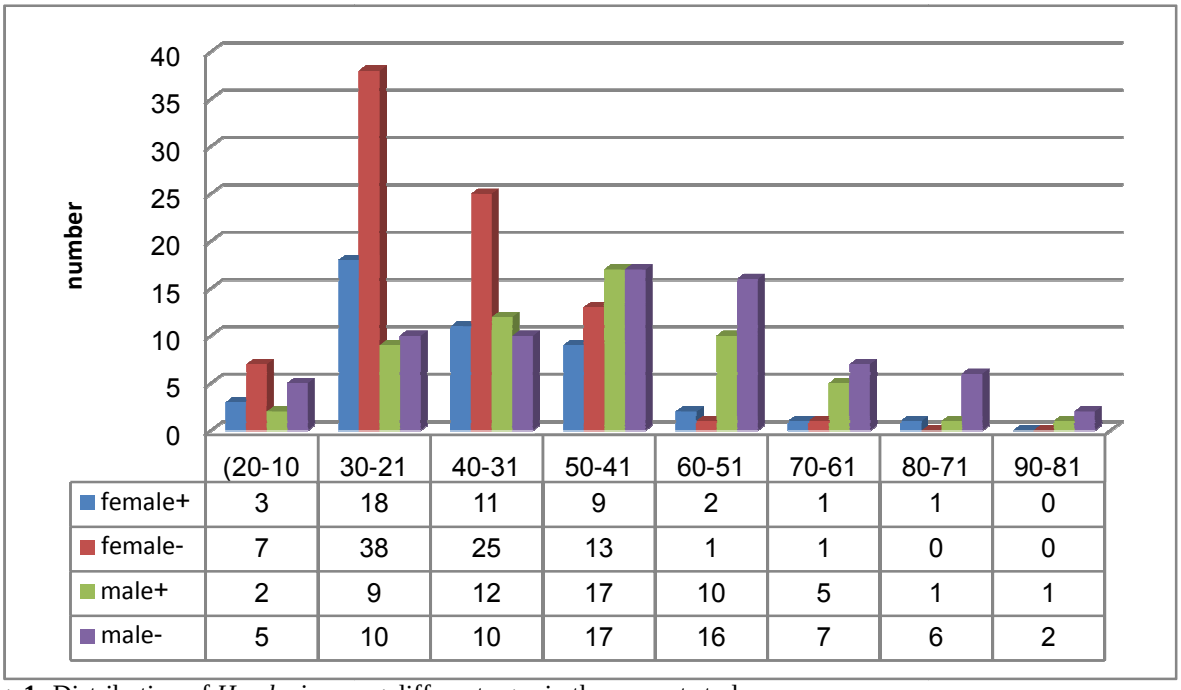
Results:

Rapid ECOtest :

In this test, serum sample was considered positive byappearance of red color in test line (T) as compared with control (C) (figure 1).The results showed that the overall percentage of infected people was 39.2% (102\260)distributed as 57\130(43.8%) infected male which was higher than the female ratio when it was 45\130(34.6%). There were 35\102 (34.3%) dark read rapid results while 67\102 (65.7%) were light red delayed results in the current *EOStest.*

Fig. 1: Rapid*ECOtest*to detect *H.pylori* infection .Strong positive results appeared in samples :13, 36, 39 , Weak results : 11 and 48, while negative results : 1 and 35.

Figure (1) shows the distribution ofthe infection according to age and gender. The highest infected aged group in male appeared between 41-50 followed by 40-31 yearswhen the rates were 17\57(29.8%) and 12\57(21.1%) , respectively .For women ,the results showed that the highest infected aged group were 21-30 year and 31-40 and the rates were 18\45(40%) and 11\45(24.4%),respectively.



	(20-10	30-21	40-31	50-41	60-51	70-61	80-71	90-81
female+	3	18	11	9	2	1	1	0
female-	7	38	25	13	1	1	0	0
male+	2	9	12	17	10	5	1	1
male-	5	10	10	17	16	7	6	2

Fig. 1: Distribution of *H.pylori* among different ages in the current study

ELISA test:

The 102 positive samples in theprevious test were subjected to IgG-class antibodies test by ELISA. The diagnostic specificity for ELISA was 92% while the sensitivity was 94% .The interest finding was that all samples which gave rapid dark read results in*EOStest* showed very high antibodies titer even more than the highest titer of the provided positive control and most of them (25\35) were out of rang .In contrast, the faint light redresult in *EOStest* gave low antibodiestiter in ELISA.Even though, five results which considered positive in the first test appeared negative in the second one.According to ELISA, 97\102(95.1%) were positive to *H.pylori*

Histopathological study:

It was used to demonstrate *H .pylori* infection in endoscopic gastric biopsy (figure 2).The results revealed that most biopsy sections(stained with H&E) showed mucosal ulceration(Fig2-A,C&E) with either heavy acute (Fig 2-E),mild or chronic inflammatory cells infiltrate (Fig 2-A,B&D).The activity (neutrophilic infiltration) of gastritis were also noted,and some mucosal ulceration was associated with mild chronic inflammatory cell

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infiltrates and mucosal glandular hyperplasia with congestion (Fig2-C).It could be said that at least 10\35 (28.6%) of the examined stained sections showed chronic gastritis with heavy infiltrate cells,while 6\35(17.1%) with mild gastritis .The activity (neutrophilic infiltration) of gastritis and the presence or absence of mucosa-associated lymphoid tissue (MALT) were also noted. In figure (2-F), the view represent a biopsy section(400x) stained with Giemsa stain illustrating the curved rod gram negative bacteria .Atrophy of glandular mucosa caused by *H. pylori*was also noticed in a 45 aged female in the current study (Fig 2-D).It was associated with focal surface epithelial ulceration and mild chronic infiltrate cells beside chronic gastritis

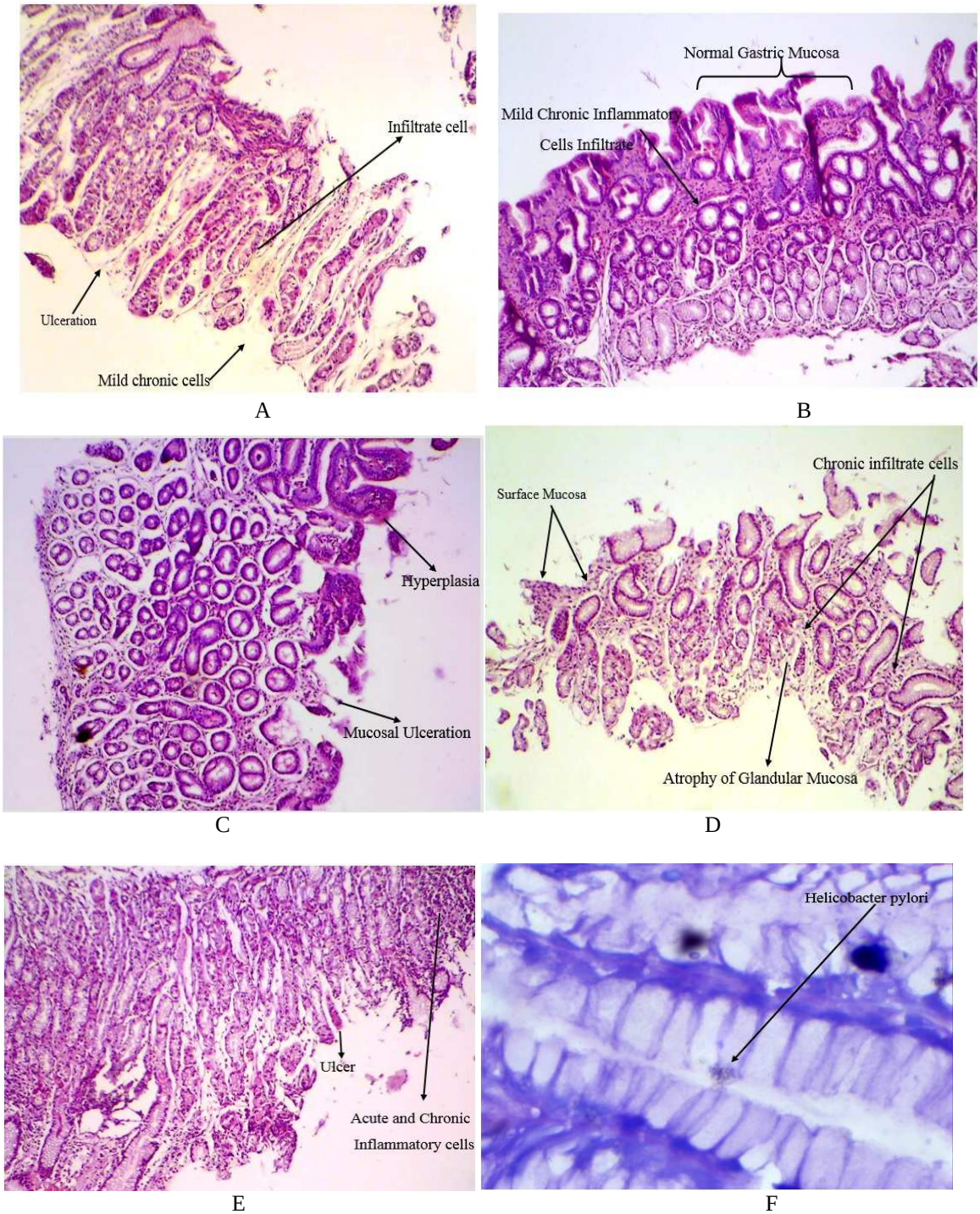

Fig. 2: Endoscopic gastric biopsy stained with H&E (200X)A: mucosal ulceration with mild chronic inflammatory cells infiltrate B :Normal looking gastric mucosa with mild chronic inflammatory cells (mild gastritis)C:surface mucosal ulceration with heavy inflammatory cells and gastric glandular hyperplasia with congestionD: superficial biopsy section showing atrophy of glandular mucosa with moderate chronic infiltrate cells E: heavy acute and chronic inflammatory cells infiltration.F: Section stained with Giemsa (400X) showing *H.pylori* inside the mucosal surface.

Discussion:

In this study, more than one method was used to check *H.pylori* in random population .Different method had been proposed to detect *H. pylori* including *H. pylori* stool antigentest,*H. pylori* breath test, Urea breath test and rapid urease test (RUT)[2,9]. Many people get *H. pylori* during childhood, but adults can get it too from food and water. It's more common in countries or communities that lack clean water or good sewage systems[2]. Beside it is possible to pick up the bacteria through contact with the saliva or other body fluids of infected people. The germs might remain in the body for years before symptoms start[1,2]. In a study done in Northern Ireland for randomly 4742selected subjects aged between 12-64 years , the overall prevalence of *H. pylori* infection was 50.5% and the prevalence increased with age from 23.4% in 12-14 years old to 72.7% in 60-64 year olds[9].In another study carried by Zhu *et al*.[10], a 5417 healthy individuals aged between 30 and 69 years old were subjected to rapid urease test and 63.41% were *H.pylori*positive which is higher than the percentage of the current study(39.2%).Besides that ,the prevalence reached peak for age group 30–39 years (90.82%) and women were more infected thus it didn't agreed with the current result.In a research carried by Miftahussurur*et al*.[11],they mentioned that there is no single test can be considered as the gold standard for the diagnosis of *H.pylori* infection and every method's has its own advantages and disadvantages.Due to the decreasing sensitivity of direct diagnostic tests several indirect tests, including antibody-based tests (serology and urine test),urea breath test (UBT), and stool antigen test (SAT) have been developed to diagnose *H.pylori* infection especially for epidemiological studies[11]. For serological diagnosis,ELISA is an easy, cheap more a current and effective method [12] .It might pointed out the increasing level of tendency of infection by reflecting antibody titer. In the current studywas pointed out that there was a correlation between the duration , deep of the color in *EOCtest*and the antibodies titer in ELISA. The more dark red color and rapid result ,the highest antibodies titer .Such results could be considered a first step for determining the tendency of infection .Stool also could be used for ELISA diagnosis before pre- treatment diagnosis of infection specially in children it will not required neither surgery nor disconcert with urea breath test[13].Other important test for *H .pylori* diagnoses is the biopsy that identify unusual changes in the mucosal layer such as Congestion, bleeding, acute or chronic infiltration cells and mucosal destruction[14].Endoscopic biopsy allows the detection of *H. pylori*, which determines the treatment for peptic ulcer disease and the presence of *H.pylori* in chronic superficial gastritis[14]. Gastritis due to *H.pylori* infection is mostly characterizes by mucosa infiltration by mononuclear cells and neutrophils as there is a strong evidence between *H.pylori* and gastritis and duodenal ulcer while moderate relation had been noticed between such infection and gastric cancer[15].Versalovic[16],demonstrated that the acute Active gastritis phase characterized by the presence of neutrophils mixed with mononuclear cells in the gastric mucosa .It might last for more than 4 weeks then it will replaced by chronic infection with neutrophils infiltration of the epithelium and lamina propria, and mononuclear infiltration associated with the development of gastric neoplasia, including gastric adenocarcinomas and gastric mucosa-associated lymphoid tissue lymphomas[16].Usually the accuracy of histological tests depend on the expertise of the pathologist and the image will reflect the evaluation of the status of the mucosa[17].Most studies Combine two methods or more to get a perfect diagnosis including serological, culture or molecular procedure to improve the accuracy of *H. pylori* detection[18].One of the characteristic feature for *H.pylori* represent by its ability to adhere to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membranecausing gastric and peptic ulceration and epithelial cell damage[19].The latency of bacteria on the gastric mucosa might turn later to active chronic gastritis specially without being treating the infection .In this study , most section showed active chronic gastritis,and this results in concrescence of other study[20].in which they demonstrated that*H.pylori* is significantly associated with active chronic gastritis and with formation of mucosa-associated lymphoid tissue (MALT).As an obligate parasites, colonization of the stomach by this bacteria might facilitate occupation of organisms similar to*H. pylori* which represent another affair from pylori infection[21]. In a study carried by Fox *et al*.[22] they mentioned that *H. pylori* infection is known to be responsible for colonization, persistence of the stomach, and triggering of inflammation, as well as the host inducing chronic gastric inflammation that progresses to atrophy, metaplasia, dysplasia, and gastric cancer .Atrophy might considered a precursor of gastric cancer and support the hypothesis that certain strains of *H. pylori* are more likely to cause gastric cancer especially those with *CagA* pathogenicity island[23]. Usually Atrophy is associated with decline in acid secretion and increasing basal serum gastrin[24]. Because of the serious complications that accompany *H.pylori* disease which might have dire consequences ,it is necessary to hold an early screening to avoid the evolution of the infection .Many specific test had been proposed weightier its serological[25]or newly developed molecular techniques like nested PCR assay to identify *H .pylori* in gastric biopsies[26], or RT PCR to monitor some mediators responsible for *H.pylori*-induced gastritis and gastric carcinogenesis[27]. Methods based molecular biology are considered highly specific and sensitive tests, and many PCR-based assays have been developed to detect *H. pylori* DNA in gastric biopsies, saliva and stool samples[28].

Conclusion:

The *EOCtest* represent simple,rapid,easyand suitable method for primary checkup diagnosis specially for random patients. Still Antibodies specific-IgG ELISA is more specific as it reflects the tendency of infection and titers of antibodies.Histopathlogical study for biopsies is reasonable for analysis to differentiate the active and chronic infections to prevent progressing of the disease.Farther work represented by molecular detection using specific housekeeping genes have been established in our lab to improve the new strategies for fast ,accurate and specific diagnosis to be compared with the current methods.

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