COMPARISON OF ^{14}C-UREA BREATH TEST WITH GASTRIC HISTOLOGY FOR DIAGNOSIS OF HELICOBACTER PYLORI INFECTION AMONG PATIENTS WITH PEPTIC ULCER DISEASE

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ABSTRACT

Background

Helicobacter pylori infection is common among patients with peptic ulcer disease. The definitive means of diagnosing infection being histology requires endoscopy and sedation making it invasive and expensive. The carbon urea breath test is generally considered to be a simple, non-invasive and accurate test for the detection of *Helicobacter pylori* infection both before and after treatment.

Objectives

Our objective was to compare histology with a less invasive and safer method, the 1^{4}C-urea breath test.

Materials and Methods

After approval of Ethical committee of the Iraqi Board for Medical Specialties, informed consent were taken from every patient before esophagogastroduodenoscopy. Fifty patients with peptic ulcer disease diagnosed by endoscopy were evaluated by ^{14}C-urea breath test. Four biopsies were taken for histology. After endoscopy, each patient was evaluated by both tests for diagnosis of *Helicobacter pylori* infection

Results

50 patients with peptic ulcer disease *Helicobacter pylori* infection was diagnosed in 35 patients (63.45%), while in 15 patients (36.55%) were negative for Helicobacter pylori infection using gastric histology and urea breath test. Compared with histology, the diagnostic values of the ^{14}C-urea breath test were: sensitivity 94%, specificity 87%, positive predictive value 94%, and negative predictive value 87%.

Conclusion

The ^{14}C-urea breath test is a noninvasive diagnostic tool for *Helicobacter pylori* infection. It's a sensitive and specific test with excellent positive predictive value and its good negative predictive value guarantee its usefulness in clinical practice.

Keywords: *Helicobacter pylori, Peptic ulcer disease, ^{14}C-urea breath test.*

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a small, curved highly motile gram negative bacillus which only infects the mucus layer of the human stomach[1, 2]. Since the discovery in 1984, this bacteria was associated with gastritis and peptic ulcer, it is recognized as being highly prevalent but this varies with age and socio-economic status[3, 4]. Infection is mainly acquired in childhood and is usually asymptomatic [5]. However in about 15–20% of subjects long-term infection can lead to peptic ulcer or gastric cancer [6, 7]. Small, uniformly sized, translucent bacterial colonies form and the organisms can be morphologically characterized by Gram stain and their typical spiral or rod shaped appearance. High power microscopy reveals that the organism has two to seven unipolar sheathed flagella which enhance its mobility through viscous solutions [8]. In addition to morphologic characterization, the organism can be biochemically characterized as catalase, oxidase, and urease positive. Urease appears to be vital for its survival and colonization; it is produced in abundance, making up more than 5 percent of the organism's total protein weight. Bacterial urease activity is clinically important because it forms the basis for several invasive and noninvasive tests to diagnose infection [8, 9].

H. pylori is the most common chronic bacterial infection in humans [10, 11]. It has been demonstrated worldwide and in individuals of all ages. Conservative estimates suggest that 50 percent of the world's population is affected. Infection is more frequent and acquired at an earlier age in developing countries compared to industrialized nations [11]. Once acquired, infection persists and may or may not produce gastroduodenal disease. In developing nations, where the majority of children are infected before the age of 10, the prevalence in adults peaks at more than 80 percent before age 50 [11, 12].

The risk of acquiring *H. pylori* infection is related to socioeconomic status and living conditions early in life. Factors such as density of housing, overcrowding, number of siblings, sharing a bed, and lack of running water have all been linked to a higher acquisition of *H. pylori* infection [13-15]. *H. pylori* transmission: Person-to-person transmission of *H. pylori* through either fecal/oral or oral/oral exposure seems most likely [16, 17].

A variety of noninvasive tests for the diagnosis of *H. pylori* are available or being evaluated. These include urea breath testing (UBT), stool antigen testing, and serology.

Urea breath testing is based upon the hydrolysis of urea by *H. pylori* to produce CO_2 and ammonia. A labeled carbon isotope is given by mouth; *H. pylori* liberates tagged CO_2, which can be detected in breath samples.[18]. The sensitivity and specificity of UBT is approximately 88 to 95 and 95 to 100 percent, respectively [19]. False positive results are therefore uncommon. False negative results may be observed in patients who are taking antisecretory therapy, bismuth, or antibiotics [20, 21].

To reduce false negative results, the patient should be off antibiotics for at least four weeks and off PPIs (proton pump inhibitors) for at least two weeks [22]. In patients hospitalized for upper gastrointestinal bleeding, early diagnosis of *H. pylori* may improve the likelihood that the patient is discharged with effective therapy. Thus, UBT should be considered as soon as the patient has begun an oral diet [23].

Serological tests to detect IgG have been most studied and IgG ELISAs are the predominant serological test available for clinical use. As a result, guidelines from the United States and Europe refer only to IgG testing [22, 24].

The presence of *H. pylori* in the stool of infected patients has led to the development of fecal assays. [25-27]. The accuracy of the fecal test was evaluated in a study involving 270 patients in whom the diagnosis of *H. pylori* was established by endoscopy and UBT, The sensitivity and specificity of the test were 94 and 86 percent [28]

The diagnosis of *H. pylori* can usually be established during endoscopy by one of three methods: biopsy urease test, histology, and less commonly bacterial culture. Choosing among these tests depends upon the clinical circumstance, the accuracy of the tests, and the relative costs [22]

Antral biopsies can be tested for urease activity using several techniques, which have similar diagnostic accuracy (commercially available kits include CLO test, PyloriTek, and Hp-fast) [21]. With this technique, one or two pieces of tissue are placed in an agar well that contains urea and a pH reagent .Urease cleaves urea to liberate ammonia producing an alkaline pH and a resultant color change .The CLO test may become positive as early as one hour after collection, but a final reading at 24 hours is recommended [29]. The sensitivity of biopsy urease tests is approximately 90 to 95 percent, and specificity is 95 to 100 percent [22]. Thus false positive tests are unusual. However, false negative results can

occur in patients with recent gastrointestinal bleeding or with the use of PPIs, H2 antagonists (histamine 2 receptor antagonist), antibiotics, or bismuth containing compounds .Obtaining tissue samples from the antrum and the fundus may increase the sensitivity of the test in these patients [30].

Rapid urease testing (RUT) kits, biopsy specimens are sandwiched between a reagent strip with a pH indicator and a pad containing urea .One hour sensitivity and specificity are comparable (89 to 98 percent and 89 to 93 percent, respectively) to those seen with agar gel tests at 24 hours and superior to agar gel tests at one hour [21, 31, 32].

Gastric biopsy can be helpful in making the primary diagnosis of *H pylori* infection .It also provides information regarding the presence of gastritis and the detection of intestinal metaplasia and mucosa associated lymphoid tissue (MALT) [22].The accuracy of histologic diagnosis of *H. pylori* infection can be improved by using special stains such as Giemsa or specific immune stains, most of research study confirm that gastric histology regards as the gold standard for diagnosis of Helicobacter pylori infection [20, 33]. Due to the patchy distribution of *H. pylori* in the gastric mucosa, tissue specimens should be obtained from different areas of the stomach [34].

The majority of patients with duodenal ulcer (DU) are infected with *H. pylori*. However, in multicenter trials, *H. pylori* was absent in almost 30 percent of patients with an endoscopically documented duodenal ulcer [35]. Studies have revealed that patients who had DU with negative *H. pylori* generally had a shorter duration of symptoms and that many had regularly used nonsteroidal anti-inflammatory drugs (NSAIDs) [36, 37]. Such patients have a significantly worse outcome , especially if treated empirically for *H. pylori* infection .Thus , *H. pylori* status should be determined in all ulcer patients before initiating treatment [37]. High acid output may be a cause of recurrent DU in patients in whom pylori has been eradicated [38]. However while the association between *H. pylori* and DU is strong, it is not specific . As examples, *H. pylori* infection is also found in patients with gastric ulcers (65 to 95 percent) [36].

Aims of the Study

1. To evaluate the frequency of *H. pylori* infection among patients with peptic ulcer disease using urea breath test and gastric biopsy.
2. To find accuracy , sensitivity , specificity , positive predictive value and negative predictive value of ^{14}C Urea breath test in comparison to gastric histology
3. To find correlation between Aspirin and NSAIDs use with *H. pylori* infection

PATIENTS AND METHODS

This prospective study was conducted in Sulaimani / Kurdistan Region of Iraq, in the Kurdistan Center for Gastroenterology and Hepatology from March 1st, 2010 to November 1st, 2010. Fifty patients with peptic ulcer disease (PUD) were included in this study all the cases of PUD were diagnosed and confirmed by endoscopic features of benign ulcers which have smooth, regular, rounded edges, with a flat, smooth ulcer base often filled with exudates, exclusion criteria included presence of malignant gastric ulcers, patient presenting with upper gastrointestinal bleeding, patient using proton pump inhibitors, or antibiotics in the preceding 4weeks and previous gastric surgery. Informed consent was obtained before entry.

Endoscopic procedures done using video endoscopic system (GIF-Q240;Olympus-Japan), patients were fasted for at least 8hours before the procedure, endoscopic procedure done under conscious sedation using Midazolam 5mg and pethidine 50 mg. Esophagogastroduodenoscopy (EGD) was used to identify PUD including gastric ulcer (GU) and duodenal ulcer (DU) with other abnormalities, at least 4biopsy specimen were taken from gastric antrum and corpus according to Sydney criteria for *H. pylori* diagnosis and were placed in 2 ml phosphate buffered saline at 4°C for bacteriological examination. Histological investigation was performed using Giemsa staining to detect *H. pylori.* Before the result was known, and within 48 hours after endoscopy, the ^{14}C-urea breath test was done.

The urea breath test was done after an overnight fast. Diagnostic test were performed by Helioprobe^{14}C-UBT (Noster System .Stockholm, Sweden). Heliprobe 1 micro Curie ^{14}C was given to the patients as either a capsule or solution in water. After 10-15 min, the patients were asked to blow into the Heliprobe breath card. A color change in breath cards indicated that a sufficient volume of CO_2 was collected .Then breath cards were analyzed by the Heliprobe analyzer (scintillation counter). Infection status was determined by the number of detected ^{14}C counts per measurement (CPM). The results were categorized in three grades

including grade 0 (CPM ≤ 50) and grade I (CPM > 50).Grade 0 was considered as negative (uninfected by H.pylori), and grade 1 as positive (infected by *H. pylori*).

Statistical analysis of the results was carried out using statistical package of social sciences (SPSS) version 15 software. Chi-square test was used for subgroup analysis. The p <0.05 was considered statistically significant.

RESULTS

In this study 50 patients with peptic ulcer disease were enrolled, 27 (54%) patients were male, and 23 (46%) patients were females .The age was between 15 to 81 years with mean age of 46.6 years (SD ±17.1). Frequency and percent of clinical symptoms in the study cases reveals that the most common clinical presentation were dyspepsia (54%) followed by dyspepsia and vomiting (32%), dyspepsia and weight loss (12%) which all cases of gastric ulcer and only 1 patient (2%) presented with dyspepsia , weight loss and vomiting .The frequency of smoking history, Aspirin and NSAIDs use reveals that 26% of patients were smokers, and 40% of patients were using Aspirin and NSAIDs (16% using Aspirin and 24% using NSAIDs).UBT results were as follow: Among 43 patients with duodenal ulcer 30 patients (69.8%) UBT was positive, while 13 patients (30.2%) were negative, while among patients with gastric ulcer 4 patients (57.1%) UBT was positive while 3 patients (42.9%) were negative .

But the result of gastric histology for *H. pylori* diagnosis were as follow: Among 43 patients with duodenal ulcer 30 patients (69.8%) gastric histology was positive, while 13 patients (30.2%) were negative, while among patients with gastric ulcer 4 patients (57.1%) gastric histology was positive while 3 patients (42.9%) were negative. Two of the cases of peptic ulcer disease had positive UBT but histology was negative, and two of the cases which had negative UBT confirmed to have positive result by histology. There was statistically significant relation between the result of UBT and gastric histology for diagnosis of *H. pylori* infection (P value 0.0001) as shown in Figure 1.

The relationship between types of PUD and results of UBT with gastric histology is shown in Table -1- and it reveals that there is no statistically significant relation between types of ulcer with UBT and gastric histology types with P-value of 0.501 and 0.511 respectively.

There was no statistically significant relationship between age of patients with the types of PUD and results of UBT with gastric histology as shown in Table 2.

The relation of aspirin use with the result of UBT and histology reveals that there is statistically significant relation between aspirin use with histology results only with a P-value of 0.009. The association between UBT and the degree of gastric inflammation shows that 96% of patients with positive UBT have moderate to severe gastritis with P-value of 0.0001. The sensitivity, specificity, positive predictive value and negative predictive value of UBT for diagnosis of *H. pylori* infection was 94%,87%, 94% and 87% respectively.

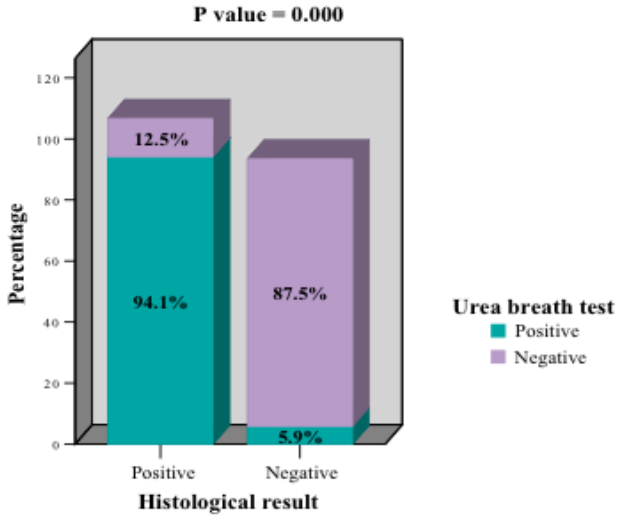

Figure 1. The comparison between UBT and gastric histology.

Table -1- Relation between types of PUD and UBT with gastric histology results

Variables	Peptic ulcer diseases		P value
	Duodenal ulcer N (%)	Gastric Ulcer N (%)	
Urea breath test			
Positive	30(69.8)	4(57.1)	0.501
Negative	13(30.2)	3(42.9)	
Histology			
Positive	30(69.8)	4(57.1)	0.511
Negative	13(30.2)	3(42.9)	

Table 2. Relationship between age, types of PUD and UBT with gastric histology results

Variables	Age Mean± Std. Deviation	P value
Peptic ulcer diseases		
Duodenal ulcer	46.0 ± 17.0	0.535
Gastric ulcer	50.4 ± 19.7	
Urea breath test		
Positive	43.0 ± 15.7	0.045
Negative	52.9 ± 18.5	
Histology		
Positive	43.9 ± 16.3	0.111
Negative	52.3 ± 18.2	

DISCUSSION

This study was performed in a random group of patients with suspected PUD referred to Gastroenterology center in Sulaimani–Iraq. Our present study focuses on the frequency of *H. pylori* infection among patients with PUD and the comparison of accuracy, sensitivity, specificity, positive predictive value and negative predictive value ^{14}C- UBT with gastric histology .

In this study we found that among 50 patients with PUD; 27 were male (54%)and 23 (46%) were female and this observation is comparable to a study done by Kurata, [39] who found that PUD still is predominant in males. Our data also similar to Lam, [40] study who confirmed male predominance of PUD. While Yasuyuki Gotto[41] found that the prevalence of *H. pylori* infection was higher in women 16.4% compared with men 10.6% but this study conducted among healthy individuals.

In the current study we found that 43 patients (86%) were diagnosed with duodenal ulcer while 7 patients (14%) were diagnosed with gastric ulcer and this in close to the conclusion of Singh, V et al [42] who found 12:1 predominance of DU over GU, also this is similar to the study done by Wong, et al [43] who found that DU were more common than GU.

In total of 50 patients with PUD, 34 patients (63.4%) using gastric histology and using UBT, had *H. pylori* infection positive results and this conclusion is close to the conclusion of Ciociola, et al [35] who found that 73% of DU patients were infected with *H. pylori*. Jyotheeswaran, et al [44] also found that 61% of patients with PUD were infected with *H. pylori*.

Our result is also similar to a study done in Iran by Nakhaei, [45] who found that the prevalence of *H. pylori* infection was 70% among patients with PUD. Chiorean, et al [46] also found similar rates of *H. pylori* infection among patients with PUD of 64%.

In this study there is history of NSAIDs usage in 12 patients (24%) and aspirin use in 8 patients (16%) and

this finding important as there is synergism between *H. pylori* and NSAIDs in producing both peptic ulcer and ulcer bleeding [47].

Currently, the UBT is considered to be one of the most important and reliable non-invasive methods for diagnosis of *H. pylori* infection. In this study we found that the sensitivity and specificity, positive predictive value and negative predictive value of ^{14}C-UBT for diagnosis of *H. pylori* infection in comparison with gastric histology was 94%, 87%, 94% and 87% respectively (P value of 0.0001) this result is very close to study result done by Howden, et al [19] who found the sensitivity and specificity of UBT is approximately 88% to 95% and 95% to 100%, respectively.

UBT is a highly sensitive and specific test compared to invasive methods used for *H. pylori* diagnosis .The sensitivity and specificity of ^{14}C- UBT were estimated 98% and 91% by Rasool et al [48].Gomollon et al [49], reported that the UBT test was much more sensitive (97%) and specific (100%) than other conventional tests including enzyme immune assay (EIA), rapid urease test, histology and culture. A meta analysis by Zhou et al revealed that ^{14}C-UBT showed a diagnostic sensitivity of 96% and specificity of 93%[50]

In this study we found the urea breath test had well correlated with the inflammation of gastric mucosa (P < 0.0001) .as in 96% of patient with positive UBT there was moderate to severe gastritis and this conclusion is similar to Mion et al [51] study who found similar results.

In conclusions, *H. pylori* infection is the most common cause of peptic ulcer disease .The ^{14}C- urea breath test is a noninvasive diagnostic tool for *H. pylori* infection; it is comparable with histology in diagnosing *H. pylori* infection among patients with PUD. It is sensitive, specific with excellent positive predictive value and its good negative predictive value guarantees its usefulness in clinical practice and it is well correlated with the degree of gastric inflammation.

We recommend using UBT for diagnosing *H. pylori* infection in communities with high prevalence of gastric cancers and MALT lymphoma patients and to confirm successful eradication and also in those who are about to use aspirin and NSAIDs to predict the occurrence of adverse upper GI disorders specially bleeding upper GI lesions and to decide on the preventive prophylactic use of PPI in *H. pylori* infection patients.

Limitations of the study

Small sample size and unavailability of previous studies regarding UBT in our region.

REFERENCES

1. Marshall BJ, Warren JR, Goodwin CS. Duodenal ulcer relapse after eradication of Campylobacter pylori. Lancet 1989; i: 846.

2. Jones DM, Lessels AM, Eldridge J. Campylobacter like organisms in the gastric mucosa: Culture, histological and serological studies. J ClinPathol 1984; 37: 1002–1006.

3 .Logan RPH, Walker MM. Epidemiology and diagnosis of Helicobacter pylori infection. Br Med J 2001; 323: 920–922.

4 .Whitaker CJ, DubielAJ.Gaplin.Social and geographical risk factors in Helicobacter pylori infection.Epidemiol Infect 1993; 111: 63.

5 .Neale KR, Logan RPH.The epidemiology and transmission of Helicobacter pylori infection in children.AlimentPharmacol 1995; 9(Suppl 2): 77–84.

6. NIH Consensus Development Panel on Helicobacter pylori in peptic ulcer disease. Helicobacter pylori in peptic ulcer disease.JAMA 1994; 272: 65–69.

7. International Agency for Research on Cancer. Schistosomes, liver flukes and Helicobacter pylori. Lyon: IARC, 1994; 61: 177–241.

8. Goodwin, CS, Worsley, BW. Microbiology of Helicobacter pylori. GastroenterolClin North Am 1993; 22:5.

9. Amieva, MR, El-Omar, EM. Host-bacterial interactions in Helicobacter pylori infection. Gastroenterology 2008; 134:306.

10. Cave, DR. Transmission and epidemiology of Helicobacter pylori. Am J Med 1996; 100(Suppl 5A):12S.

11. Pounder, RE, Ng, D. The prevalence of Helicobacter pylori infection in different countries. AlimentPharmacolTher 1995; 9(Suppl 2):33.

12. Torres, J, Leal-Herrera, Y, Perez-Perez, G, et al. A community-based seroepidemiologic study of Helicobacter pylori infection in Mexico. J Infect Dis 1998; 178:1089.

13. Hunt, RH, Sumanac, K, Huang, JQ. Should we kill or should we save Helicobacter pylori? Aliment PharmacolTher 2001; 15 Suppl 1:51.

14. Webb, PM, Knight, T, Greaves, S, et al. Relation

between infection with Helicobacter pylori and living conditions in childhood: Evidence for person to person transmission in early life. Br Med J 1994; 308:750.

15. Kivi, M, Johansson, AL, Reilly, M, et al. Helicobacter pylori status in family members as risk factors for infection in children. Epidemiol Infect 2005; 133:645.

16. Perry, S, de la, Luz Sanchez M, Yang, S, et al. Gastroenteritis and transmission of Helicobacter pylori infection in households. Emerg Infect Dis 2006; 12:1701.

17. Megraud, F. Transmission of Helicobacter pylori: Fecal-oral versus oral-oral route. Aliment PharmacolTher 1995; 9(Suppl 2):85.

18. Leide-Svegborn, S, Stenstrom, K, Olofsson, M, et al. Biokinetics and radiation doses for carbon-14 urea in adults and children undergoing the Helicobacter pylori breath test. Eur J Nucl Med 1999; 26:573

19. Howden, CW, Hunt, RH. Guidelines for the management of Helicobacter pylori infection. Am J Gastroenterol 1998; 93:2330.

20. Gatta, L, Vakil, N, Ricci, C,et al. Effect of proton pump inhibitors and antacid therapy on C urea breath tests and stool test for Helicobacter pylori infection. Am J Gastroenterol 2004; 99:823.

21. Laine, L, Estrada, R, Trujillo, M, et al. Effect of proton-pump inhibitor therapy on diagnostic testing for Helicobacter pylori. Ann Intern Med 1998; 129:547.

22. Chey, WD, Wong, BCY. American College of Gastroenterologoy guideline on the management of Helicobacter pylori infection. Am J Gastroenterol 2007; 102:1808.

23. Gisbert, JP, Esteban, C, Jiminez, I, et al. 13C-urea breath test during hospitalization for the diagnosis of Helicobacter pylori infection in peptic ulcer bleeding. Helicobacter 2007; 12:231.

24. Malfertheiner, P, Megraud, F, O'Morain, C, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 2007; 56:772.

25. Kelly, SM, Pitcher, MC, Farmery, SM, et al. Isolation of Helicobacter pylori from feces of patients with dyspepsia in the United Kingdom. Gastroenterology 1994; 107:1671.

26. Braden, B, Teuber, G, Dietrich, CF, et al. Comparison of new faecal antigen test with ^{13}C-urea breath test for detecting Helicobacter pylori infection and monitoring eradication treatment: Prospective clinical evaluation. BMJ 2000; 320:148.

27. Vaira, D, Malfertheiner, P, Megraud, F, et al. Diagnosis of Helicobacter pylori infection with a new non-invasive antigen-based assay. Lancet 1999; 354:30.

28. Trevisani, L, Sartori, S, Galvani, F, et al. Evaluation of a new enzyme immunoassay for detecting Helicobacter pylori in feces: A prospective pilot study. Am J Gastroenterol 1999; 94:1830.

29. Siddique I, Al-Mekhaizeem, K, Alateeqi,N,etal. Diagnosis of Helicobacter pylori :improving the sensitivity of CLO testby increasing the number of gastric antral biopsies . J Cin Gastroenterol2008 ;42:356

30. Weston, AP, Campbell , DR, Hassanein, RES, et al .Prospective multivariate evaluation of CLO test performance .Am J Gastroenterol 1997;92:1310

31. Yousfi, MM, El-Zimaity,MTGenta,RM et al .Evaluation of a new reagent strip rapid urease test for detection of Helicobacter pylori infection .GastrointestEndosc 1996;44:519

32. Nishikawa, K, Sugiyama, T, Kato, M et al. A prospective evaluation of a new rapid urease tests before and after eradication treatment of helicobacter pylori, in comparison with histology, culture and C-urea breath test.GastrointestEndosc 2000; 51:164

33. Wright , CL,Kelly ,JK, The use of routine special stains for upper gastrointestinal biopsies .Am J SurgPathol 2006;30:357.

34. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ and Bosques-Padilla FJ. (2014). A review of elicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 20(6), 1438–1449.

35. Ciociola ,AA, McSorlwy,DJ,Turner,KG,et al .Helicobacter pylori infection rates in duodenal ulcer patients in the United States may be lower than previously estimated .Am J Gastroenterol1999; 94:1834.

36.Tytgat, Langenberg, W, Rauws, E, et al.Compylobacter-like organism (CLO) in the human stomach.Gastroenterology1985; 88:1620.

37. Harris,AW,Gummett,PA,Phull,PS,et al .Recurrence of duodenal ulcer after Helicobacter pylori eradication is related to high acid output.AlimentPharmacolTher 1997;11:331.

38. McColl, KE,El-Nujumi, AM,Chitajulla,RS, et al .A study of the pathogenesis of Helicobacter pylori-negative duodenal ulceration.Gut 1993;34:762.

39. Kurata, JH. Epidemiology: peptic ulcer risk factors. SeminGastrointest Dis 1993; 4:2.

40. Lam, SK. Differences in peptic ulcer between East and West. Baillieres Best Pract Res ClinGastroenterol 2000; 14:41.

41.Goto Y, Syam AF, Darnindro N et al .Prevalence and Risk Factors for Helicobacter Pylori Infection among Healthy Inhabitants in Northern Jakarta, Indonesia. Asian Pac J Cancer Prev.2016 Oct 1; 17(10):4747-4753

42. Singh, V, Trikha, B, Nain, CK, et al. Epidemiology of Helicobacter pylori and peptic ulcer in india. J GastroenterolHepatol 2002; 17:659.

43. Wong, BC, Ching, CK, Lam, SK, et al. Differential north to south gastric cancer-duodenal ulcer gradient in China. China Ulcer Study Group. J GastroenterolHepatol 1998; 13:1050.

44. Jyotheeswaran, S, Shah, AN, Jin, HO, et al. Prevalence of Helicobacter pylori in peptic ulcer patients in greater Rochester, NY: Is empirical triple therapy justified? AM J Gasteroenterol 1998; 93:574 .

45. Nakhaei, MM. Prevalence of helicobacter pylori infection in patients with digestive complaints using urea breath test in Mashhad, Northeast Iran. JRHS. 2010; 10(2):77-80.

46. Chiorean, M, Locke, G, Zinsmeister, A, et al. Changing rates of Helicobacter pylori testing and treatment in patients with peptic ulcer disease. Am J Gastroenterol 2002; 97:3015.

47. Huang, J, Sridhar, S, and Hunt, R. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet 2002; 359:14.)

48. Rasool S, Abid S, Jafri W. Validity and cost comparison of 14carbon urea breathe test for diagnosis of H.pylori in dyspeptic patients. World J Gastroenterol. 2007; 13(6):925-929.

49. Gomollon F, Ducons JA, Santolaria S, et al. Breath test is very reliable for diagnosis of Helicobacter pylori infection in real clinical practice. Digestive and Liver Disease. 2003; 35(9):612-618.)

50.Zhou Q, Li L, Ai Y et al Diagnostic accuracy of the 14C-urea breath test in Helicobacter pylori infections: a meta-analysis.Wien KlinWochenschr. 2017 Jan; 129(1-2):38-45

51. Mion F, Delecluse HJ, Rousseau M, et al [13C-urea breath test for the diagnosis of Helicobacter pylori infection. Comparison with histology]. GastroenterolClin Biol. 1994; 18(12):1106-11.