Sensitivity and specificity of Rapid Urease test (RUT) in diagnosis of H. Pylori, in patients presenting with symptoms of acid peptic disease at a tertiary care hospital.

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How to citation this article: Dr. Anand P. Zingade, Dr.Toshit K. Lodha, Dr. Balaji D. Dhaigude, Dr. Shaunak S. Tuvar, "Cytochrome C as a marker of Apoptotic Cell Death after Chemo-Radiotherapy in Locally Advanced Head and Neck Cancer Patients", IJMACR- May - 2023, Volume – 6, Issue - 3, P. No. 149 – 157.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Helicobacter Pylori is a microaerophilic fastidious human pathogen that has been implicated in acid-peptic diseases of the stomach and duodenum and neoplasm of the stomach. Rapid Urease Test (RUT) is an indirect test of the presence of H. Pylori based on the presence of urease in or on the gastric mucosa. Several factors have been studied that may cause alterations in the sensitivity and specificity of RUT.

Objective: To find sensitivity and specificity of rapid urease test to diagnose H. Pylori infection.

Materials And Methods: This is a prospective observational study done over a period of 24 months from December 2020 to November 2022 by including all patients presenting to opd with symptoms of acid peptic disease above 18 years of age with written and informed consent. All data analysis has been done using SPSS version 22 software.

Results: Out of 80 patients a maximum number of patients was in the age group of 41-50 years (28.7 %). Males were most commonly (55%) affected as compared to females (45%). It was observed that rapid urease tests and histopathological findings showed a strong association with statistical significance. ($P<0.001$). The sensitivity of RUT was 91.49%, specificity of 90.91% with accuracy of 91.25%. Out of a total of 47 H. Pylori positive patients, 43 were correctly diagnosed by RUT, while 4 patients were false negative With RUT, owing to multiple factors such as prior smoking, alcohol consumption and prior proton pump inhibitors consumption.

Conclusion: RUT is accurate for the diagnosis of H. Pylori infection and it is as sensitive and specific as histopathological examination, although, some factors may affect sensitivity and specificity.

Keywords: H. Pylori., Rapid Urease Test.

Introduction

H. Pylori: General Facts

H. pylori is a Gram-negative, helical-shaped bacteria, and has a tuft of 5–7 polar flagella located at its distal end. H. pylori is microaerophilic and has its optimal growth conditions at 5–19% O2, 5–10% CO2, 37° C, and high humidity; similar to conditions that are found in the gastric mucosa. Although being a gastric pathogen, the high acidity of the stomach juice is lethal for the bacteria. H. pylori, therefore, resides in the slimy mucus layer that lines and protects the epithelial cells from the acidic gastric juice. [1]
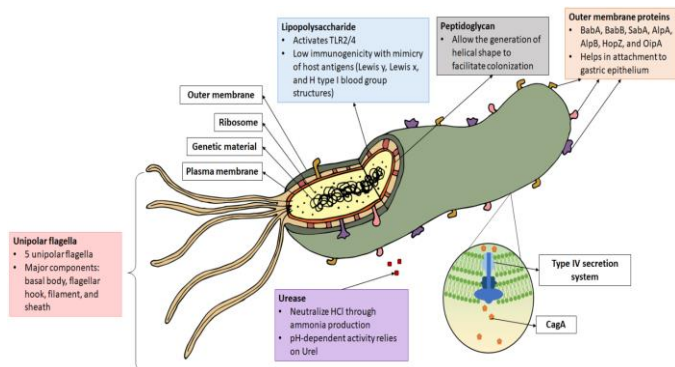

Figure 1

H. pylori colonizes the stomach and, in particular, the less acidic antrum. During disease progression, pH initially decreases due to hypersecretion and H. pylori might move into the first part of the intestine, the duodenum. This region is less resistant to infection and peptic ulcer could develop.

Urease

In addition to the chemotactic and swimming capabilities that H. pylori utilize to escape the acidic gastric juice, it also possesses the cytoplasmic enzyme urease. Urease is a large 1.1-MDa complex that neutralizes the local environment by converting urea to NH3 and HCO3-. NH3 neutralizes protons in the periplasm and HCO3- acts as a buffer to maintain a pH of 6.1.[2] Lysis of bacterial cells in the surrounding releases urease and buffers into the microenvironment of the live bacteria, thus the bacterial cells are buffered both internally and externally.[3] This neutralization of the mucin also changes its rheological properties and makes it easier for H. pylori to penetrate.[4]

Diagnostic Methods for H. Pylori Infection

Invasive methods

Histology

H. pylori has a characteristic spiral appearance making it relatively easy to identify during histological evaluation. It can be seen on Hematoxylin-Eosin, Warthin-Starry or modified Giemsa staining. Histology also gives additional information about the grade of inflammation in the gastric mucosa by evaluating the infiltration of granulocytes and lymphocytes as well as the presence of gastric atrophy and intestinal metaplasia. Biopsies need to be taken both from the antrum and the corpus and at least two biopsies are needed from each location.[5] Specificity is high but sensitivity can vary due to the patchy distribution of the bacteria.[6,7]

Culture

Biopsies from the gastric mucosa are cultivated in a microaerophilic environment for up to seven days. Colonies of bacteria can typically be seen after 4-5 days. The colonies are identified as H. pylori by the typical appearance of the colonies and by urease-, catalase- and oxidase tests and Gram-staining. The accuracy of H.pylori culture is considered to be high and it is dependent on the laboratory settings and low-temperature transportation of the samples.[7]

Rapid urease test

Biopsies from the gastric mucosa are placed in a gel containing urease and a pH-dependent colour indicator. If the sample contains active H. pylori, its urease will convert urea to ammonia and bicarbonate which will raise in pH and lead to a colour change in the gel from yellow to red. The sensitivity and specificity are both about 95 % respectively. If the patient is infected with H.pylori, the test will be positive in 3 hours in 90% of the cases and the final evaluation is possible after 24 hours.[8]

Figure 2

False-negative and false-positive results

RUT is a test for the presence of the urease enzyme. The actual results will however depend on the gastric disease and the likelihood of atrophic changes or exogenous factors that reduce the bacterial load and thus produce false negative results. False positive results can occur if other urease-containing organisms are present in sufficient quantity or if one allows contact with the specimen and the media for a prolonged period, typically longer than 24 hours. As noted before, approximately 105 bacteria must be present in the biopsy sample for a positive result and anything that reduces the bacterial density such as the use of antibiotics, bismuth-containing compounds, or proton pump inhibitors may result in false-negative results.[9,10]

The two most common reasons for false negative results are the recent use of proton pump inhibitors and the presence of intestinal metaplasia. H2-receptor antagonists do not reduce bacterial density and can be used up to the day of the test.[11] It is unclear how long one must wait after stopping proton pump inhibitors before the possibility of a false negative result becomes unlikely.

False positives are rare and when present may be due to the presence of other urease-containing organisms such as Proteus mirabilis, Citrobactor freundii, Klebsiella pneumonia, Enterobacter cloacae and Staphylococcus aureus. However, unless the patient has achlorhydria or hypochlorhydria, non-H. pylori organisms are unlikely to be present in sufficient concentration to produce a positive test unless the RUT substrate lacks an inhibitor to bacterial growth which they may possibly overgrow during the 24-hour observation period. [12]

Material And Methods

Study Design: The present prospective observational study was undertaken to role of the Rapid urease test in the diagnosis and treatment of H. pylori in patients presenting with symptoms of Acid Peptic Disease at a tertiary care hospital. The present study period was from December 2020 to November-2022. The study population was patients presenting with acid peptic symptoms attending the hospital.

Sample Size: A total sample size of 80 patients presenting with acid peptic symptoms attending the hospital was included in the study by following inclusion and exclusion criteria.

Sample size Estimation:

n = (Zα/2+Zβ)2 * (p1(1-p1)+p2(1-p2)) / (p1-p2)2,

Assuming 90% sensitivity, the required sample size = 71

Adding a 10% dropout rate, the required sample size will be = 71+8 =79,Minimum.

Hence, the minimum sample size of 80 patients presenting with OPD with symptoms of Acid Peptic Disease was studied.

All Data were double entered using Microsoft Excel 2007 and analyzed using SPSS version 22. Data were summarized in frequency tables, pie charts and histograms. Categorical variables were reported as a proportion. Continuous data were described as means (standard deviation) or medians (interquartile range) depending on the distribution of data. The T-test was applied in the following results whenever necessary.

Observations And Results

Table 1: Age Distribution among patients

Age	Frequency	Percentage
20-30	06	7.6
31-40	19	23.7
41-50	23	28.7
51-60	20	25.0
61-70	12	15.0
Total	80	100.0

The above table shows age distribution among patients. The maximum number of patients were in the age group of 41-50 years (28.7%), followed by in 51-60 years (25%). The mean age among the distribution of cases was 43.73 ±10.12 years.
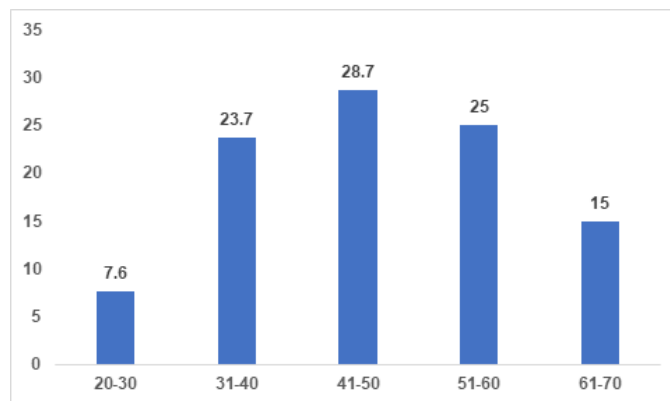

Table 2: Gender Distribution

Gender	Frequency	Percentage
Male	44	55.0
Female	36	45.0
Total	80	100.0

The above table shows distribution patients on basis of sex. Out of 80 patients, males (55%) were the most commonly affected when compared to females (45%)
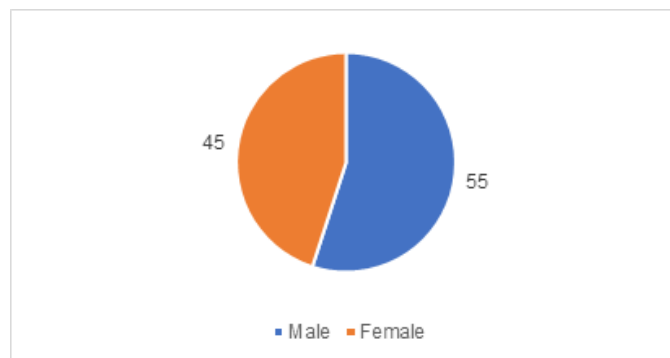

Table 3: Alcohol consumption distribution among patients:

Alcohol	Frequency	Percentage
Yes	19	23.7
No	61	76.3
Total	80	100.0

The above table shows distribution patients on basis of alcohol consumption. Out of 80 patients, 19 (23.7%) patients had history of alcohol consumption.
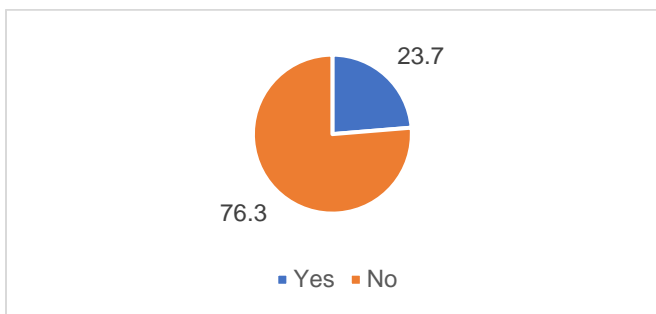
Table 4: Smoking distribution among patients:

Smoking	Frequency	Percentage
Yes	23	28.7
No	57	71.3
Total	80	100.0

The above table shows distribution patients on basis of smoking. Out of 80 patients, 23 (28.7%) patients had history of smoking.
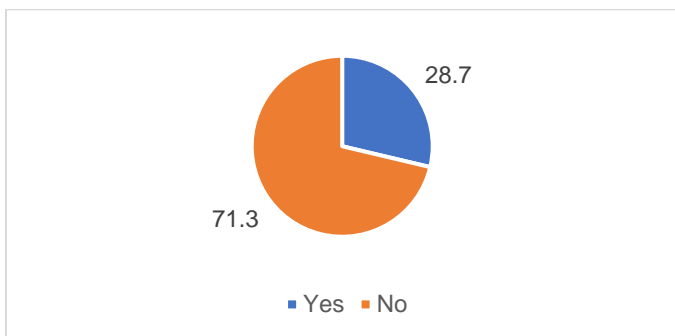

Table 5: Proton pump inhibitor consumption distribution among patients:

Proton pump inhibitor consumption	Frequency	Percentage
Yes	21	26.25
No	59	76.75
Total	80	100.0

The above table shows the distribution of patients on the basis of consumption of proton pump inhibitors. Out of 80 patients, 21 (26.2%) patients had a history of consumption of proton pump inhibitors.
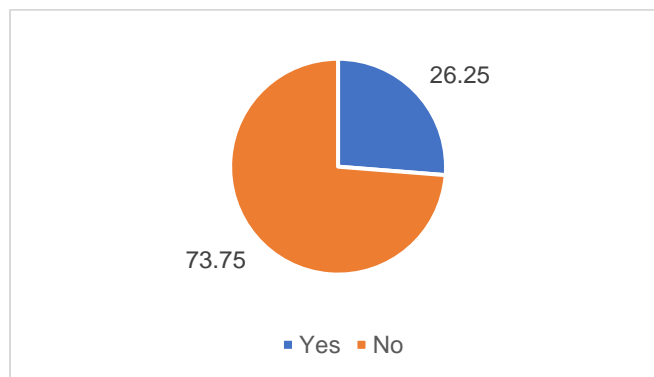

Table 6: Rapid urease test result distribution

Rapid urease test	Frequency	Percentage
Positive	46	57.5
Negative	34	42.5
TOTAL	80	100.0

The above table shows distribution patients according to rapid urease test. Out of 80 patients, 43 (57.5%) patients had Rapid urease test positive.
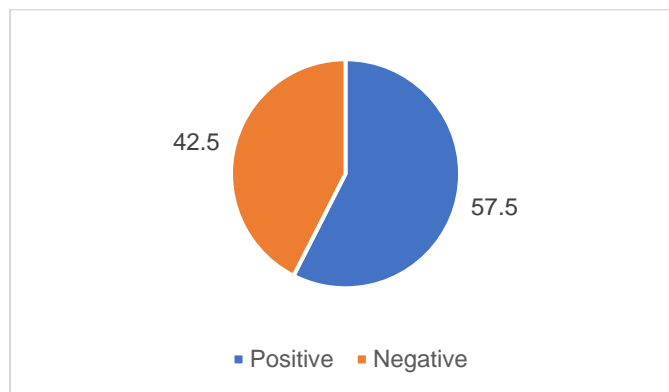

Table 7: Endoscopic findings among study subjects

Endoscopic findings		No of patients (n=80)	Percentage
Esophagus	Normal	71	88.75
	Erosion/ Inflammation	07	8.75
	Ulcer	02	2.50
Stomach	Normal	43	53.75

	Erosion/ Inflammation	22	27.5
	Ulcer	11	13.75
	Growth	04	5.00
Duodenum	Normal	51	77.5
	Erosion/ Inflammation	14	17.5
	Ulcer	04	5.00

The above table shows endoscopic findings among patients. It was observed that among 80 patients, in the esophagus 7 (8.75%) patients had inflammation while 2 (2.5%) had ulcers. In the stomach 22 (27.5%) patients had inflammation while 11 (13.75%) had ulcers and 4 (5%) patients had growth. In duodenum, 14 (17.5%) patients had inflammation while 4 (5%) had ulcers.
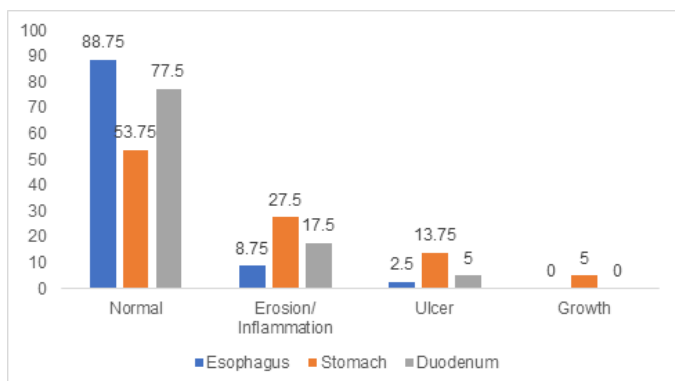

Table 8: Histopathological findings among study subjects:

Histopathological findings	No of patients (n=80)*	Percentage
Chronic inflammation	61	76.25
Acute inflammation	20	25.00
Glandular atrophy	03	03.75
Intestinal metaplasia	02	02.50
Lymphoid aggregates	17	21.25

*Multiple findings

The above table shows histopathological findings among patients. It was observed that among 80 patients, 61 (76.25%) patients had chronic inflammation while 20 (25%) had acute inflammation. Glandular atrophy among 3 (3.75%) patients, intestinal metaplasia in 2 (2.5%) and lymphoid aggregates in 17 (21.25%) patients.
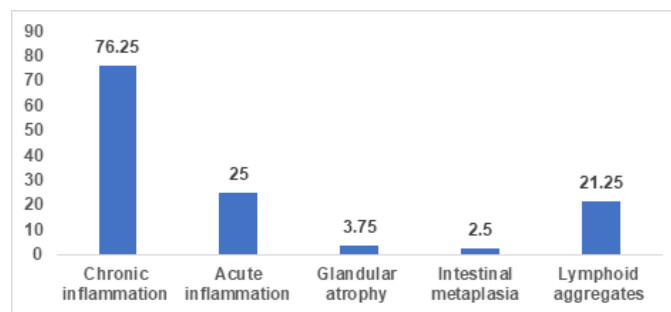

Table 9: Distribution according to H. Pylori infection by Histopathological findings:

H. Pylori	No of patients	Percentage
Positive	47	58.75
Negative	33	41.25
Total	80	100

The above table shows distribution patients according to histopathological findings. Out of 80 patients, 47 (58.75%) patients had H. Pylori positive histopathological findings.
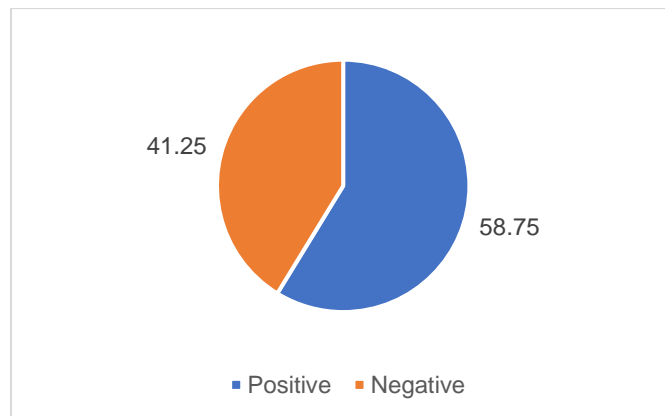
Table 10: Correlation of Rapid urease findings and H&E findings:

Findings		H & E			P value*
		Positive	Negative	Total	
Rapid Urease test	Positive	43	03	46	<0.0001 (S)
	Negative	04	30	34	
	Total	47	33	80	

The above table shows relation of rapid urease test and histopathological findings among patients. It was observed that rapid urease test and histopathological findings showed strong association with statistical significance. (P<0.001)

The sensitivity of rapid urease test was 91.49%, specificity of 90.91% with accuracy of 91.25%.
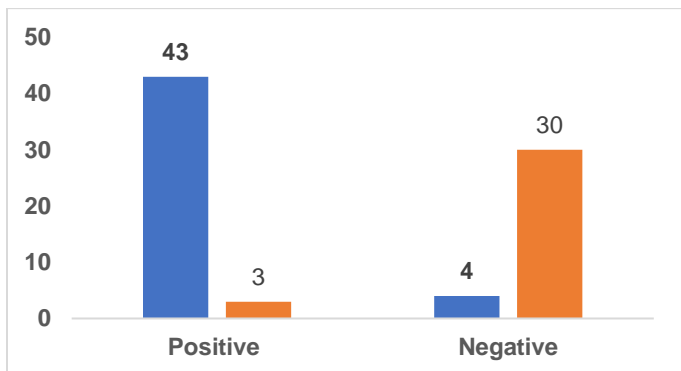
Rapid urease test

Statistic	Value	95% CI
Sensitivity	91.49%	79.62% to 97.63%
Specificity	90.91%	75.67% to 98.08%
Positive Predictive Value (*)	93.48%	82.92% to 97.69%
Negative Predictive Value (*)	88.24%	74.49% to 95.07%
Accuracy (*)	91.25%	82.80% to 96.41%

Discussion

- The RUT is the most frequently used test for diagnosis of H. Pylori in routine Upper GI endoscopy. As the results are obtained bedside immediately during endoscopy, it is extremely valuable as the specific treatment is initiated prior to histopathological examination.

- Out of 80 patients, 19 (23.7%) patients had a history of alcohol consumption.

- Out of 80 patients, 23 (28.7%) patients had a history of smoking.

- In the present study, out of 80 patients, 21 (26.25%) patients had a history of consumption of proton pump inhibitors.

- Javed Yakoob et al [127] in a study on the role of rapid urease test observed 22% (57/109) were not on any medications while 48% (52/109) used PPI before presentation to the outpatients.

- The distribution of patients according to the rapid urease test showed out of 80 patients, 46 (57.5%) patients had a Rapid urease test positive.

- Javed Yakoob et al [13] in a study on the role of the rapid urease test observed rapid urease test was positive in 40% (44/109) and negative in 60% (65/109).

- Alireza Eslaminejad et al [14] studied the best accuracy of RUT observed concerning RUT, 102 (69.9%) showed negative results after 5 minutes compared to 44 (30.1%) positive cases of H.pylori.

- Virendra S. Athavale et al [15] studied the sensitivity and specificity of the rapid urease test (RUT) and observed 84 patients (84%) were detected positive by the RUT method.

- The relation of rapid urease test and histopathological findings among patients showed that rapid urease test and histopathological findings showed a strong association with statistical significance. (P<0.001)

- The sensitivity of the rapid urease test was 91.49%, specificity of 90.91% with accuracy of 91.25%.

- This is comparable with a study conducted by Jemilohun et al[137] found sensitivity, specificity,

PPV and NPV were 93.33%, 75.6 %, 80.76 % and 91.17 % respectively.

- Virendra S. Athavale et al [15] studied the sensitivity and specificity of rapid urease test (RUT) and observed there was an association between RUT and HPE finding in a study group with the sensitivity being 96.38 % and specificity being 74.47 %, PPV was 95.24% and NPV was 81.25%. There was an association between RUT and HPE finding at follow up with the sensitivity being 88.89 % and specificity being 100%, PPV was 100% and NPV was 98.73%.

- Javed Yakoob et al[13] in a study on the role of rapid urease test observed the sensitivity, specificity, PPV, NPV and like-hood ratio of a positive and negative Pronto Dry test with and without PPI were 43.3%, 86.4%, 81.3%, 3.18, 0.656 and 52.8% vs 71.9%, 80%, 82.1%, 69%, 3.59 and 0.35.

Limitations

1. Single centre study

Conclusion

The present study concludes that RUT is quick, accurate, sensitive and specific for the detection of H. Pylori with comparable results to histopathological examination.

References

1. Pounder RE, Ng D (1995). The prevalence of H. pylori infection in different countries. Aliment Pharmacol Ther; 9 (suppl. 2): 33-39.

2. Boyanova, L., Mitov, I. & Vladimirov, B., 2011. Helicobacter Pylori, Caister Academic Pr. 2011.

3. Dunn, B.E. et al., 1997. Localization of Helicobacter pylori urease and heat shock protein in human gastric biopsies. Infection and immunity, 65(4), pp.1181–1188.

4. Celli, J.P. et al., 2009. Helicobacter pylori moves through mucus by reducing mucin viscoelasticity. Proceedings of the National Academy of Sciences of the United States of America, 106(34), pp.14321–14326.

5. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161-81.

6. Leodolter A, Wolle K, Malfertheiner P. Current standards in the diagnosis of Helicobacter pylori infection. Dig Dis. 2001;19:116-22.

7. Sipponen P. Update on the pathologic approach to the diagnosis of gastritis, gastric atrophy, and Helicobacter pylori and its sequelae. J Clin Gastroenterol. 2001;32:196-202.

8. Vaira D, Holton J, Menegatti M, Ricci C, Gatta L, Geminiani A, et al. Review article:invasive and non-invasive tests for Helicobacter pylori infection. Aliment Pharmacol Ther. 2000;14 Suppl 3:13-22.

9. Graham DY, Lew GM, Malaty HM, et al.Factors influencing the eradication of Helicobacter pylori with triple therapy. Gastroenterology 1992;102:493-6.

10. Graham DY, Opekun AR, Hammoud F, et al.Studies regarding the mechanism of false negative urea breath tests with proton pump inhibitors. Am J Gastroenterol 2003;98:1005-9.

11. Attumi TA, Graham DY. Follow-up testing after treatment of Helicobacter pylori infections: cautions, caveats, and recommendations. Clin Gastroenterol Hepatol 2011;9:373-5.

12. Osaki T, Mabe K, Hanawa T, et al.Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of Helicobacter pylori infection. J Med Microbiol 2008;57:814-9.

13. Yakoob J, Jafri W, Abid S, Jafri N, Abbas Z, Hamid S, Islam M, Anis K, Shah HA, Shaikh H. Role of rapid urease test and histopathology in the diagnosis of Helicobacter pylori infection in a developing country. BMC gastroenterology. 2005 Dec;5(1):1-4.

14. Eslaminejad A, Marashian SM, Aboutorabi M, Sadr M, Agah S. Determination of optimal time for reading of rapid urease test diagnosis of Helicobacter pylori. Gastroenterol Hepatol Bed Bench. 2020 Summer;13(3):232-237.

15. Athavale VS, Singh V, Khandalkar SN, Nirhale DS, Lad A, Shetty I. Comparative study of rapid urease test and histopathological examination for detection of H. pylori infection. International Surgery Journal. 2017 Nov 25;4(12):4071-5.