Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

ORIGINAL RESEARCH ARTICLE

Role of Various Staining Method in Detection of H. Pylori Infection in Gastric Lesions

Dr. Malabika Mahato,
Assistant Professor, Department of Pathology, Andaman and Nicobar Islands Institute of Medical Sciences, Port Blair, Andaman and Nicobar Island, India.

Received: 18-09-2024 / Revised: 27-10-2024 / Accepted: 05-11-2024

ABSTRACT

INTRODUCTION
H. pylori infection is a major cause of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric B-cell lymphoma (MALT lymphoma). Infection with this organism affects more than half the world's population. Although, several special stains have been used to detect H. pylori in histological sections; however, their sensitivity and specificity vary greatly.

AIMS AND OBJECTIVES
This study was conducted to compare the role of various staining methods like Hematoxylin and eosin (H&E), Giemsa and Warthin starry stain (WSS) in diagnosing H. pylori in chronic gastritis.

MATERIALS AND METHODS
This is a prospective study, includes 50 gastric biopsies with histopathological diagnosis of chronic gastritis. Slides were stained with Hematoxylin and eosin (H&E), Giemsa and Warthin starry stain. Sensitivity of H&E, Giemsa and Warthin starry stain was calculated.

RESULTS
Most the biopsies were obtained from gastric antrum by endoscopic method. Recurrent abdominal pain was the most frequently encountered clinical presentation. H.Pylori was identified in 62% biopsies stained by H&E, followed by 64% of biopsies stained with Giemsa stain, the frequency of detection was greater with WSS stained sections which is 68%.

CONCLUSION
Although H & E and modified Giemsa stains, are standard stains for detection of H. pylori; the reliability and yield is better using Warthin starry stains; especially when present even in coccoid forms or in small numbers.

KEYWORDS
H. Pylori, Special Stains, Chronic Gastritis.

INTRODUCTION
Helicobacter pylori (H.Pylori) is a Gram-negative, spiral, microaerophilic human pathogen and have shown very good relationship with different gastro duodenal diseases. It is one of the more frequent chronic infections in human since the isolation of the pathogen.[1-3] Barry Marshall and Robin Warren of Perth, Western Australia discovered H. pylori in 1982.[4-6] Since its discovery H.Pylori has been researched widely in the pathology and microbiology fields. However, when it is cultured

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

on solid medium, the bacteria assume a rod-like shape. H. pylori are 2.5 to 5.0 mm long and 0.5 to 1.0 mm wide.

Currently, over half of the global population is colonized with H.pylori, which is the most important cause of chronic inflammation and peptic ulcer.[7,8] H. pylori infection even recognized as prominent causative role for gastric cancer; which is being the first most frequent cancer and the next most important reason for cancer related mortality globally (WCRF, 1997).[9]

Typically, all Helicobacter species express urease enzymes, an enzyme that is necessary for microbial survival.[10,11] Experimental deletion of the gene urease renders H. pylori is not able to colonize gastric mucosa. Urease is an intracellular enzyme that is bound to the outer layer of other bacterial membrane upon bacterial lyses.[12]

The source or sources of H. Pylori transmission route is limited. The route of transmission is combinations of oral-oral, gastro-oral or faecal oral because of absence of access to purified drinking water and proper sanitation.[13] The NIH (National Institute of Health) Consensus Development Panel on Helicobacter Pylori in peptic ulcers recommends that the diagnosis should be established before therapy is started.[14-16]

There are various diagnostic methods to develop to test H. pylori that display varying accuracy, specificity and feasibility for the utilization in medical practice or in research. The various tests have various advantages and disadvantages, and the option of test(s) to be used is dependent on many factors like clinical or research use. In general the tests need either an intervention namely biopsy from an endoscopic examination, or a peripheral test such as blood investigation to diagnose HP antibodies, or antigen and/or antibodies tests from samples from saliva, urine and faeces, or checking the urea in expired breath air.

Histology is generally considered to be the gold standard in the direct investigation of H. Pylori infection and it is even the initial technique employed for the investigation of H. pylori. Though, numerous factors affect the detecting accurateness of histology, namely site, size and number of biopsies, methods of staining, proton pump inhibitor (PPI), antibiotics, knowledge and experience of the examining pathologist.[17,18] Histological diagnosis of the microorganism has usually been considered to have lower sensitivity at 80% - 95% with 100% specificity. Microscopical assessment of a stained smear made of crushed tissue is convenient and quick. Staining is the crucial portion of Histopathological investigation and various stains namely regular HE staining, Giemsa, Warthin-Starry, Hp silver stain, toluidine blue, acridine orange, McMullen, Genta, Dieterle, and immunohistochemical stain employed to identify H. pylori. If carried out skilfully, Warthin-Starry silver stain as originally used by Warren gives the best results. Modified Giemsa staining is most favorable investigation for the preponderance of the investigators due to its ease of performance. The sensitivity of modified Giemsa stain is 85%. Though, the specificity of modified Giemsa stain depends on the structural appearance. The specificity of immune-staining permits detection of low numbers or even single organism.[19]

MATERIAL AND METHODS

This study was carried out in the Department of Pathology, Sri Balaji Medical College and Hospital, Chennai for a period from November 2016 to October 2018. This study protocol was accepted by Ethical review Committee of Sri Balaji Medical College. This study was planned as Prospective study and includes 50 gastric biopsies with histopathological diagnosis of chronic gastritis.

Inclusion Criteria

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

Subjects with several symptoms confined to upper GIT advised by the physicians for endoscopic examination and subsequently diagnosed as individuals with gastritis, peptic ulcer (PU) were incorporated in this study.

Exclusion Criteria

Patients taking H. pylori eradication treatment and consumption of antibiotics, proton pump inhibitors, bismuth containing compounds or sucralfate during the 4 weeks preceding the endoscopy were excluded in the study.

Staining Methods

Hematoxylin & Eosin Stain

Section: Paraffin

Procedure

1. Wax removal -10-15 minutes
2. Xylene 1 and Xylene 2 for 10 minutes each
3. Put in acetone – 10 dips each
4. Washed in water for 2 minutes
5. Harris hematoxylin is put and left for 8-10minutes
6. Wash under tap water for 2 minutes
7. Dipped in 1% acid alcohol -1 dip
8. Then wash under running water for 5-8 minutes
9. Dipped in Eosin -2 dips
10. Wash with running water
11. Let it dry for 2-3 minutes
12. Mount with DPX (Distyrene, plasticizer, and xylene)

Interpretation

i. **H.pylori -** stained as blue to purple rods
ii. Nucleus - stained blue to purple colour
iii. Connective tissue and cytoplasm - stained pink colour

Giemsa Staining Technique

Procedure

1. Dewax in Xylol 1 and Xylol 2 – 10minutes each
2. Two dips in acetone
3. Wash under running tap water
4. 1 drop of Giemsa, 9 parts water (1:10 dilution) is placed on the section.
5. Wait for 20 minutes
6. Wash with tap water
7. Dehydrate, clear in xylene and mount in DPX.

Interpretation

Microorganism – dark blue
Background- pink pale blue

Warthin Starry Staining

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

Warthin-starry is a silver based stain used in histopathology for visualization of Helicobacter pylori.

Solution Preparation

1. Acetate buffer pH 3.6
2. 1% silver nitrate solution in pH 3.6 acetate buffer
3. Developer solution:
   2% silver nitrate = 15ml
   5% gelatine solution = 3.75 ml
   15% hydroquinone = 12ml

Procedure

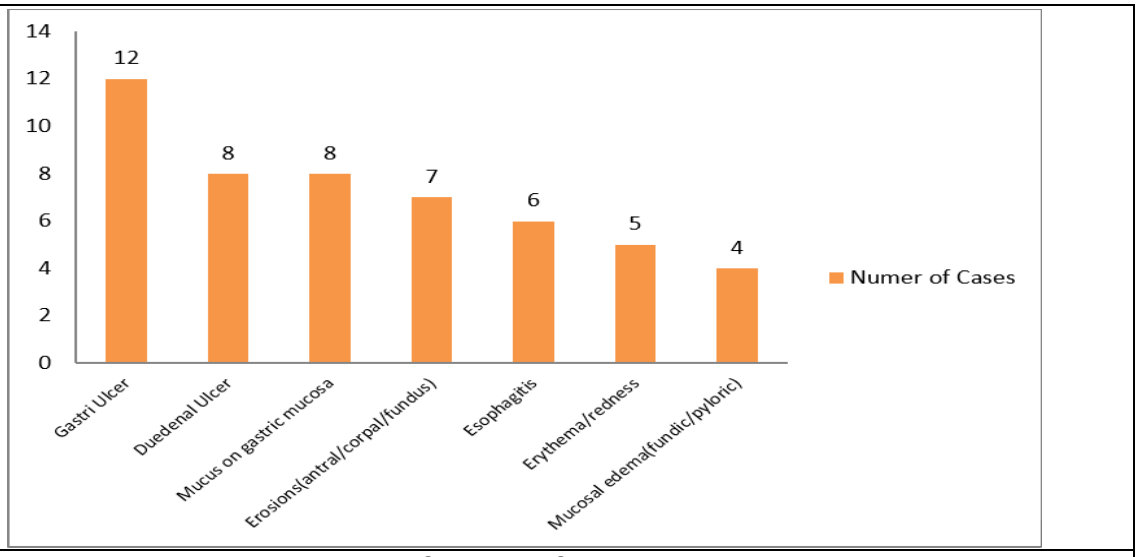
1. Deparaffinize and bring sections to distilled water.
2. Place slides in 1% silver nitrate solution for 45 seconds in microwave. Let stand for 1 minute at room temperature.
3. Prepare and preheat developer solution in water bath.
4. Remove slides of silver solution. Do not rinse. Place slides horizontally on a slide rack and cover with developer solution. Allow sections to develop, until they are light yellow to golden brown, approximately 1 min or less.
5. Wash quickly in hot tap water
6. Rinse in distilled water
7. Dehydrate, clear and mount

Interpretation

Helicobacter pylori: Black
Back ground: Golden yellow

OBSERVATION AND RESULTS

Among 50 cases maximum number of patients presented with Gastric ulcer (12) followed by duodenal ulcer (8), gastric mucosa (8) and erosions (7), esophagitis (6), erythema (5) and mucosal edema (4).



Graph 1: Clinical findings of 50 symptomatic cases

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

Sl. No	Endoscopic Findings	Number of Cases
1	Gastric Ulcer	12
2	Duodenal Ulcer	8
3	Mucus on gastric mucosa	8
4	Erosions (antral/corpal/fundus)	7
5	Esophagitis	6
6	Erythema/redness	5
7	Mucosal edema(fundic/pyloric)	4
	Total	50
Table 1: Clinical findings of 50 symptomatic cases		

Test results	Symptomatic group (n=50)	Sensitivity	Specificity
H&E stain of biopsy specimen Positive	31 (a)	62%	*Could not be done
H&E stain of biopsy specimen Negative	18 (b)		
Total	50 (a+b)		
Table 2: Sensitivity of H&E stain			

*This test was not performed in asymptomatic group due to invasive procedure.
a= True positive
c= False negative
Sensitivity = a/a+c × 100

Table shows sensitivity of H&E stain of biopsy specimen (62%). Specificity could not be carried out as this test was not performed in asymptomatic group due to invasive procedure.

Sensitivity of Giemsa Stain in 50 Symptomatic Cases

Test results	Symptomatic group (n=50)	Sensitivity	Specificity
Gimsa stain of biopsy specimen Positive	32 (a)	64%	*Could not be done
Gimsa stain of biopsy specimen Negative	18 (b)		
Total	50 (a+b)		
***Table 3:* Sensitivity of Giemsa stain**			

* This test was not performed in asymptomatic group due to invasive procedure.
a= True positive
c= False negative
Sensitivity = a/a+c × 100

Table 3 shows the sensitivity of direct microscopy of gastric biopsy specimen on modified Giemsa stained smears which is 64%. Specificity could not be carried out, as this test was not performed in asymptomatic group due to invasive procedure.

Sensitivity of Warthin Starry Stain in 50 Symptomatic Cases

Test results	Symptomatic group (n=50)	Sensitivity	Specificity
WSS of biopsy specimen Positive	34 (a)	68%	*Could not be done
WSS of biopsy specimen Negative	17 (a)		
Total	50 (a+b)		
Table 4: Sensitivity of WSS			

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

* This test was not performed in asymptomatic group due to invasive procedure.
a= True positive
c= False negative
Sensitivity = a/a+c × 100

Table 4 shows sensitivity of WSS of biopsy specimen (RUT) 68%. Specificity could not be carried out as this test was not performed in asymptomatic group due to invasive procedure.

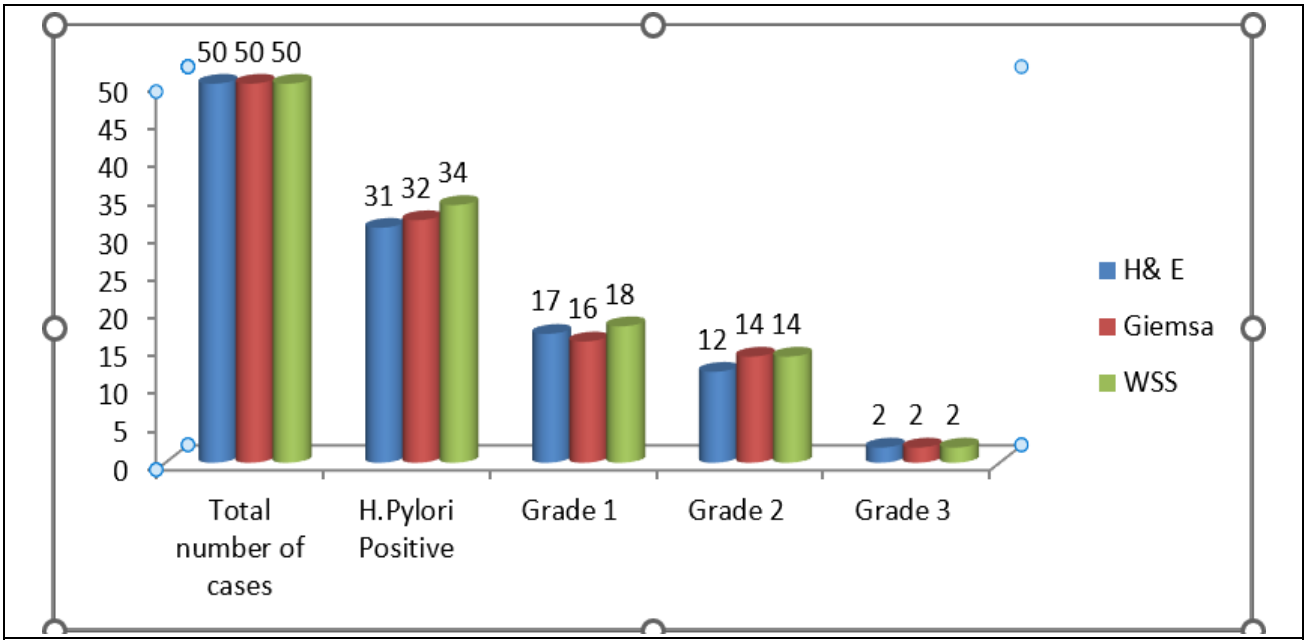
Sydney score	Activity	Chronic inflammation	Intestinal metaplasia	Atrophy	H.Pylori
1	39	29	4	13	18
2	11	17	2	0	12
3	0	4	0	0	3

Table 5: Sydney scoring system in gastric biopsy

Grading of H.Pylori Infection in Gastric Biopsy Using Sydney Scoring System in VARIOUS Staining Methods like H&E, Giemsa and WSS

Staining method	Total number of cases	H.Pylori Positive	Grade 1	Grade 2	Grade 3	H.Pylori Negative
H& E	50	31 (62%)	17	12	2	19 (38%)
Giemsa	50	32 (64%)	16	14	2	18(36%)
WSS	50	34 (68%)	18	14	2	16(32%)

Table 6: Scoring of H.Pylori Infection in Gastric biopsy



Graph 2: Scoring of H.Pylori Infection in Gastric biopsy

Table No 6 shows higher sensitivity (68%) of WSS stain in finding the grade-1, 2, 3 H.pylori positive from biopsy specimen followed by Giemsa (64%) and H&E (62%) stains. Overall when comparing with other stains in this study WSS stains shows higher sensitivity in finding the positive H.pylori cases in biopsy sample.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

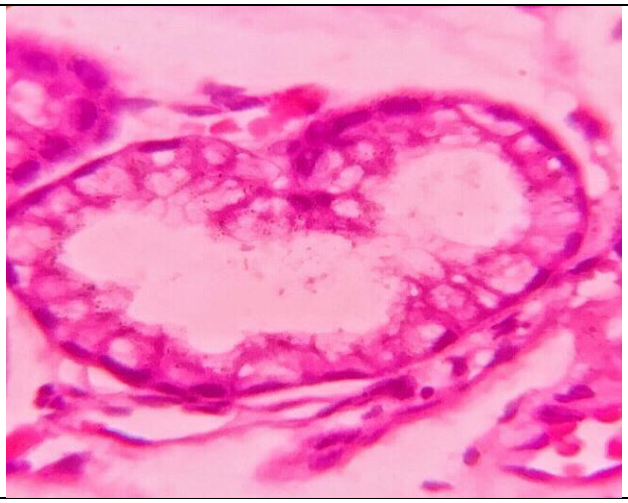


Image 1: H & E (100X), showing H.pylori in the luminal surface (grade 1 colonisation)

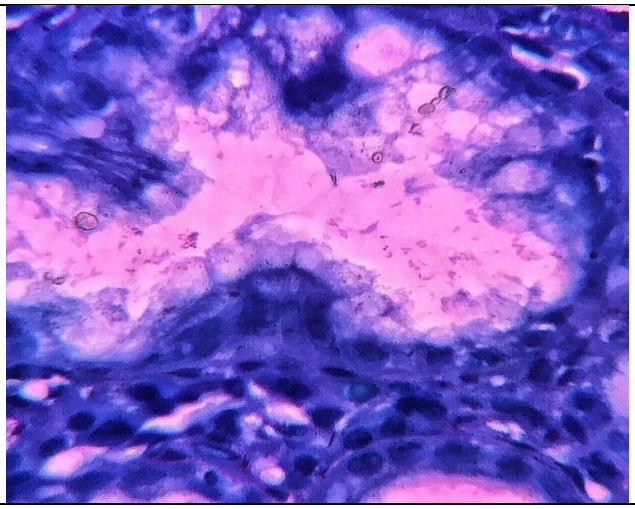


Image 2: Giemsa, (100x), showing grade 2 colonisation

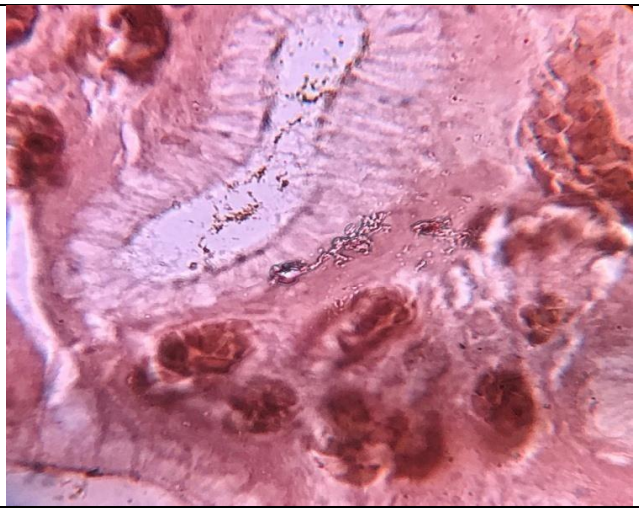


Image 3: WSS (100x), showing H.pylori in the luminal surface

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

DISCUSSION

H. pylorus is a gram negative bacterium that colonizes the human stomach resulting in chronic gastritis. The severity of the inflammation is likely to underlie H. pylori related diseases.

Presently numerous investigative procedures present for identifying H. pylori infection. Every procedure contains its own merits, demerits and limitations such as indication, sensitivity, specificity**.** Accurate investigation is required for the effective management of infections due to this organism. In the clinical situation, a quick and price effective investigative procedure for analyzing of H. pylori infection is desirable.[20,70] Accurate investigation is required for the effective management of infections due to this organism. In the clinical situation, a quick and price effective investigative procedure for analyzing of H. pylori infection is desirable.[21] There are various procedures for the examination of H. pylori and can be divided into 2 types, such as invasive procedure that needs endoscopy or the noninvasive procedure where endoscopy is not required. In the regular clinical investigations the rapid urease test, histopathological investigations, urea breath test, serology, bacterial culture and stool antigen test are most helpful procedures to investigate H.pylori infection. In our study, H&E stain, Giemsa stain and WSS stain is used as diagnostic tools to identify H.Pylori.

In this current study we found WSS stain is better in terms of sensitivity comparing with Giemsa and H&E stains. WSS shows 68% sensitivity which higher than Giemsa 64% and H&E 62%.

Our sensitivity results are in accordance with the study carried out by **Jhala et al (**100%), and **Poddar et al** (98%) who reported WSS stain has higher sensitivity among other two stains.[22,23]

From a practical point of view, we can say that identification is relatively easy with all the staining methods, but much easier with the Warthin-Starry method because the silver coating makes the organism larger. With Warthin-Starry, the H. pylori were visualized not only on the surface of the foveolar epithelium but also deep inside the gastric pits.

CONCLUSION

From the results provided in present study, the precision of the investigation for H. pylori detection can be given in order as follows: WSS>Giemsa>H&E. However, the arrangement might change slightly among similar studies. In general, the point is that in majority of studies the biopsy based investigations are chosen over other procedures and no methods can be considered as the gold standard alone.

A reliable stain to identify H.pylori infection is crucial, but none of the stains available is appropriate for all situations, each having its own drawbacks and pitfalls. In our study the results concluded that the combination of two or more stains including special stains may improve the precision of the H. pylori detection.

REFERENCES

[1] Dutta D, Clevers H. Organoid culture systems to study host–pathogen interactions. Curr Opin Immunol 2017;48:15-22.
[2] Kumari PR. A study of Helicobacter pylori in gastroduodenal diseases. Int J Curr Microbiol Appl Sci 2014;11(3):753-71.
[3] Ogihara A, Kikuchi S, Hasegawa A, Kurosawa M, Miki K, Kaneko E, et al. Relationship between Helicobacter pylori infection and smoking and drinking habits. J Gastroenterol Hepatol 2000;15(3):271-6.
[4] Marshall B, Azad M. Q&A: Barry Marshall. A bold experiment. Nature 2014;514(7522):S6-7.
[5] D'Elios MM. Helicobacter pylori, the story so far]. Med Secoli 2007;32(21):102-6.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 12, 2024

[6] Sipponen P, Price AB. The Sydney System for classification of gastritis 20 years ago. Journal of Gastroenterology and Hepatology (Australia) 2011;26:31-4.
[7] Kusters JG, Van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev 2006;19(3):449-90.
[8] Cover TL, Blaser MJ. Helicobacter pylori in health and disease. Gastroenterology 2009;136(6):1863-73.
[9] Wroblewski LE, Peek RM, Wilson KT. Helicobacter pylori and gastric cancer: Factors that modulate disease risk. Clin Microbiol Rev 2010;3(4):713-39.
[10] Ahmed N, Tenguria S, Nandanwar N. Helicobacter pylori--a seasoned pathogen by any other name. Gut Pathog 2009;1:24.
[11] Exner MM, Doig P, Trust TJ, Hancock REW. Isolation and characterization of a family of porin proteins from Helicobacter pylori. Infect Immun 1995;63(4):1567-72.
[12] Carlini CR, Ligabue-Braun R. Ureases as multifunctional toxic proteins: a review. Toxicon. 2016;110:90-109.
[13] Vale FF, Vítor JMB. Transmission pathway of Helicobacter pylori: Does food play a role in rural and urban areas? International Journal of Food Microbiology 2010;138(1-2):1-2.
[14] Gralnek I, Dumonceau JM, Kuipers E, Lanas A, Sanders D, Kurien M, et al. Diagnosis and management of nonvariceal upper gastrointestinal hemorrhage: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy 2015;47(10):a1-46.
[15] Gralnek IM, Dumonceau JM, Kuipers EJ, Lanas A, Sanders DS, Kurien M, et al. Diagnosis and management of nonvariceal upper gastrointestinal hemorrhage: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy 2015;
[16] Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, et al. Second Asia-Pacific Consensus Guidelines for Helicobacter pylori infection. J Gastroenterol Hepatol 2009;24(10):1587-600.
[17] Patel SK, Pratap CB, Jain AK, Gulati AK, Nath G. Diagnosis of Helicobacter pylori: What should be the gold standard? World J Gastroenterol. 2014;20(36):12847–59.
[18] Ochola LB, Vounatsou P, Smith T, Mabaso MLH, Newton CRJC. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. Lancet Infect Dis 2006;6(9):582-8.
[19] Gabr NS, Abdellatif MZM, Abd El-Hafeez E, Abd Rabou RAM. Comparison between ELISA and various stains techniques in laboratory diagnosis of cryptosporidiosis. J Egypt Soc Parasitol 2014;44(2):509-16.
[20] Malik GM, Mubarik M, Kadla SA. Helicobacter pylori infection in endoscopic biopsy specimens of gastric antrum: Laboratory diagnosis and comparative efficacy of three diagnostic tests. Diagnostic and therapeutic endoscopy. 1999;6(1):25-9.
[21] Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 2014;20(6):1438–49.
[22] Siegal, MD, PhD GP, Klemm, MD K, Atkinson, MD BF, Jhala, MD DN, Jhala, MD, MIAC NC. Infiltration of Helicobacter pylori in the Gastric Mucosa. Am J Clin Pathol.2003 Jan 1; 119(1):101–7.
[23] Poddar, Ujjal, and Surender Kumar Yachha. "Helicobacter pylori in children: an Indian perspective." (2007).