Comparason of different staining method for identification of Helicobacter pylori in Upper Gastro-intestinal biopsies

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Abstract: Helicobacter Pylori is a gram negative, motile microorganism that is one of the major causes of chronic gastritis. Helicobacter pylori can be detected by routine hematoxylin and eosin (H&E) stain but the detection should be supported by special stains like Giemsa stain, Warthin-Starry stain, and Modified Methylene blue and Alcian blue.Aim: This study aims to compare the results in detecting H.pylori obtained on special stains like Giemsa stain and Warthin-Starry stain along with H&E stain.
Materials and Methods: A prospective study was conducted among 50 upper GI tract endoscopic biopsies obtained from patients presenting with upper GI symptoms like dyspepsia, epigastric pain, hyperacidity at Surgery OPD in a tertiary care hospital. The sensitivity, specificity, PPV and NPV of H&E, modified Giemsa stain and Warthin-Starry stain were calculated for detection of H.pylori and comparisons were drawn.
Results: Out of the 50 cases, 27 (54%) tested positive for H. pylori and 23 (46%) were negative. Chronic gastritis was the most common histopathological diagnosis with 15 (56%) cases being H. pylori positive. Warthin-starry stain showed the highest sensitivity, specificity, PPV and NPV out of all three stains.
Conclusion: This study highlighted that although routine H&E and Giemsa staining is simple, rapid and cost-effective method in comparison to Warthin starry stain which is costlier and time consuming yet proved to be the gold standard for diagnosis of H. pylori due to its high sensitivity, specificity, PPV and NPV and good colour contrast.

Keywords: H. pylori, Modified Giemsa, Warthin-Starry, sensitivity, specificity

Introduction:

Helicobacter pylori infection and its related diseases are the most prevalent worldwide. In fact, Helicobacter was named as a "definite biological carcinogen" by the World health organisation in 1994.[1] This bacterium is Gram-negative, motile and microaerophilic and can withstand the acidity of the stomach for survival. H. pylori infection is the major cause of chronic gastritis and is important in the pathogenesis of duodenal and gastric ulcers, atrophic gastritis, MALT lymphoma and distal gastric adenocarcinoma. H. pylori was considered as 'Class I carcinogen' by International Agency for Research on Cancer (IARC).[2]
Although H. pylori may affect all ages,but older age group have more risk to infection. Ingestion of the bacteria, which is the most likely portal of entry, may occur by one or a combination of three means: oral-oral, gastro-oral, or faecal-oral, but determining a dominant route is not an easy task.

H. pylori can be detected by invasive and non-invasive tests.[3] Various non-invasive methods include 13-Carbon urea breath test (13-C-UBT), Stool antigen test and serology (Anti H. pylori IgG antibody) whereas invasive techniques include Rapid Urease Test, histopathology, culture and Polymerase Chain Reaction (PCR). The histopathological evaluation and culture are the gold standard method for detection.[4]

With the help of endoscopy, numerous small mucosal biopsies are obtained for diagnosis and monitoring the course of various gastro-intestinal lesions. For this purpose, flexible fibre optic endoscope, with an electronic video image is used. Biopsies are used for histopathological examination. In case of severe infection routine H&E can detect H. pylori bacteria in the tissue. However, with low-density of bacterial colonisation with features of chronic gastritis needs special staining for the identification of H. pylori. Hence although the organisms are often visible on Haematoxylin and Eosin (H&E) staining, but are more easily seen with Giemsa and Warthin-Starry stain.[5]The present was study aimed to do a comparative study of H&E, modified Giemsa and Warthin stain in detection of H. pylori.

Materials and Methods:

This study is a prospective study performed on the gastrointestinal endoscopic biopsies received from the department of Gastroenterology/Surgery to the department of Pathology in a tertiary care hospitalat Navi Mumbai, with asample size of 50, over a period of 15months after obtaining ethical clearance from the institution.

Inclusion criteria:

Male and female patients aged above 18 years, endoscopically established for various chronic upper abdominal symptoms including epigastric pain, dyspepsia, heartburn, nausea and vomiting from department of surgery (gastroenterology), received in the histopathology lab of department of pathology.

Exclusion criteria:

1. Anatomical abnormality of stomach
2. Patients undergoing any gastroduodenal surgery.
3. Active bleeding.
4. Patients on helicobacter pylori eradication therapy.

The study included 50 gastrointestinal biopsies from the patients who came to the department of Surgery with various chronic upper abdominal symptoms including epigastric pain, dyspepsia, heartburn, nausea and vomiting. All relevant clinical details including age, sex, clinical presentation, endoscopic findings and clinical diagnosis of patients were noted in the proforma prepared for this study.

A prior written consent for endoscopy and tissue retrieval was obtained from each patient by explaining the procedure in advance. Using Gastrointestinal videoscope Olympus GIF-H 180, Olympus GIF-V70 the endoscopy was carried out. Biopsies were taken, four biopsies from antrum, body and pylorus. The biopsies were fixed in 10% formalin; routinely processed and embedded in paraffin. The slides were stained with routine H & E, modified Giemsa and Warthin starry stain.

Results and Observations:

This randomized prospective study was conducted in Department of Pathology, MGM Medical College & Hospital, Kamothe, Navi Mumbai. This hospital is multi-specialty tertiary healthcare centre mainly catering peri urban population. During the study period of over 18 months an average of 69 suspected patients visited Department of Surgery, out of which 50 patients were included in this study from OPD as well as IPD and endoscopic biopsies were taken and submitted for examination in the Department of Pathology.

A total of 50 Upper GI endoscopic biopsies were examined, out of which 27 (54.0%) H. Pylori positive cases and 23 (46.0%) H. Pylori negative cases were identified. **(Graph 1)**

**Incidence of H. Pylori in Upper GI Endoscopic biopsies**
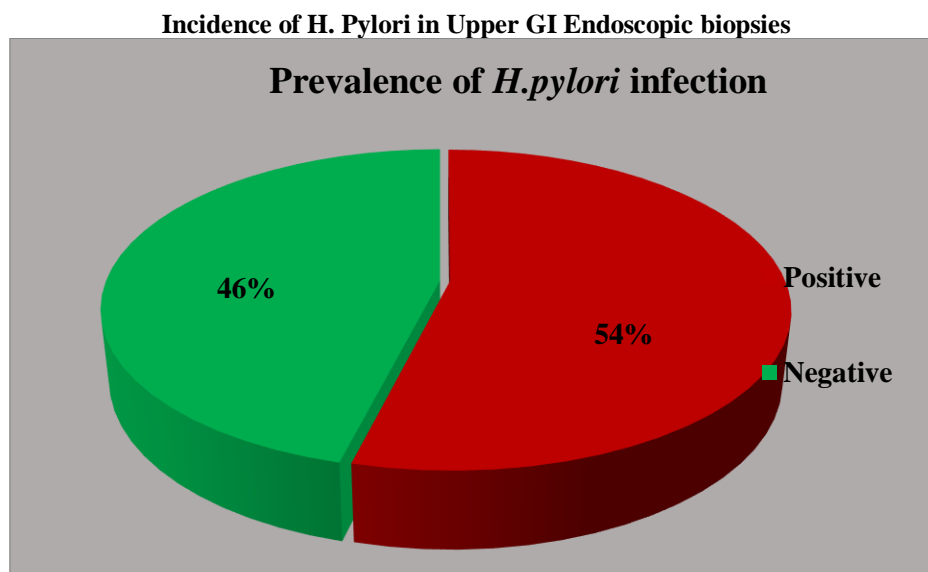
GRAPH: 1

On correlation with endoscopic and histopathological diagnosis, Gastritis (56%) was the commonest lesion in H. pylori positive cases followed by gastric erosion (22%), gastric ulcer (18%) and gastric carcinoma (4%). **(Graph 2)**
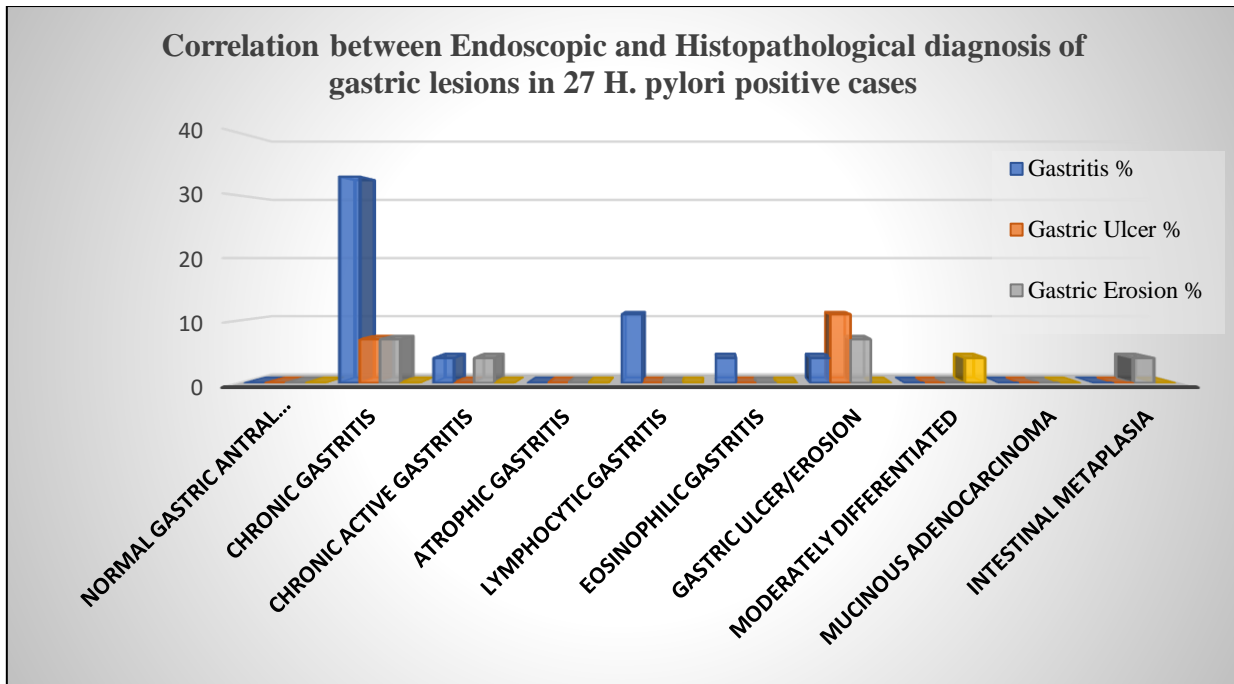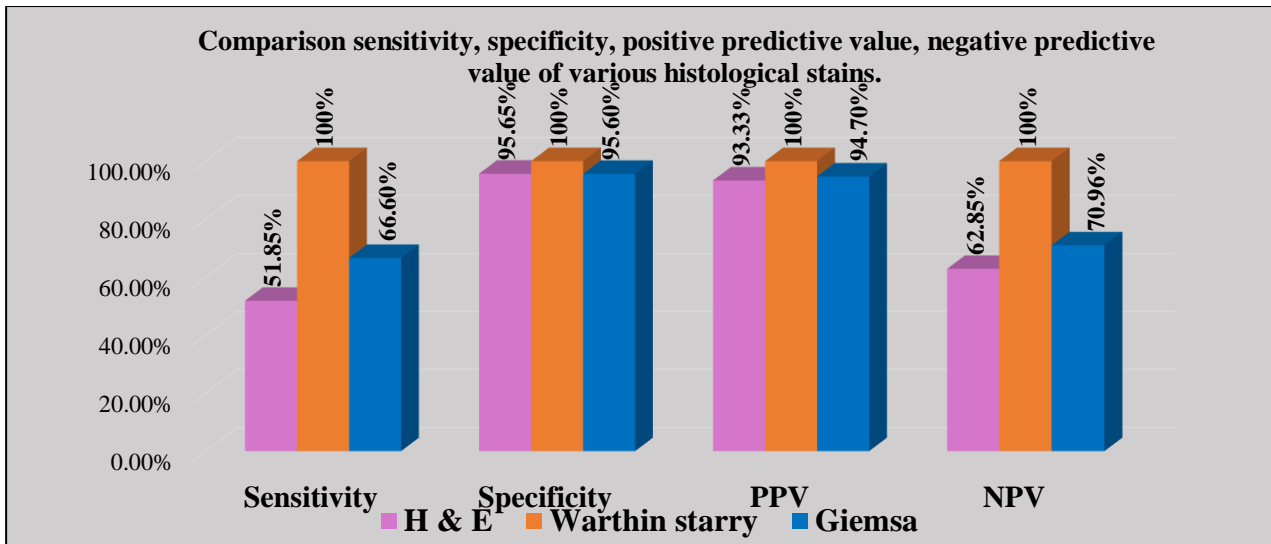
GRAPH: 2

The maximum H. pylori positive cases were identified with Warthin starry stain (54%) followed by Giemsa stain (38%), and least with H & E stain (28%). **(Graph 3)**
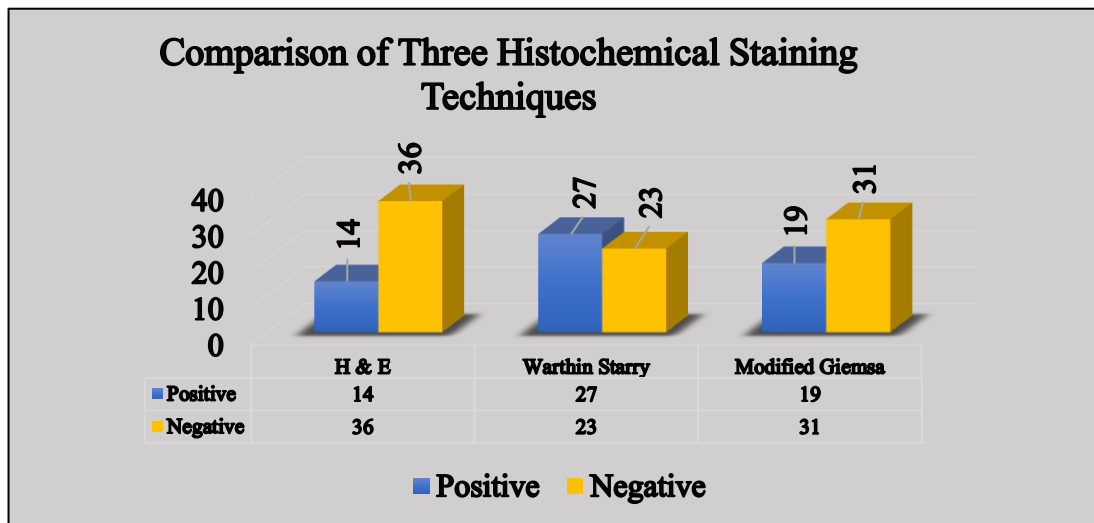
GRAPH: 3

Warthin starry stain showed highest sensitivity, specificity, PPV, NPV of 100% each and least being of H & E stain showing 51.85%, 95.65%, 93.33% and 62.85% respectively. Accuracy for the H&E, Warthin-starry and Giemsa test was observed to be 72%, 100% and 80% respectively. **(Graph 4)**
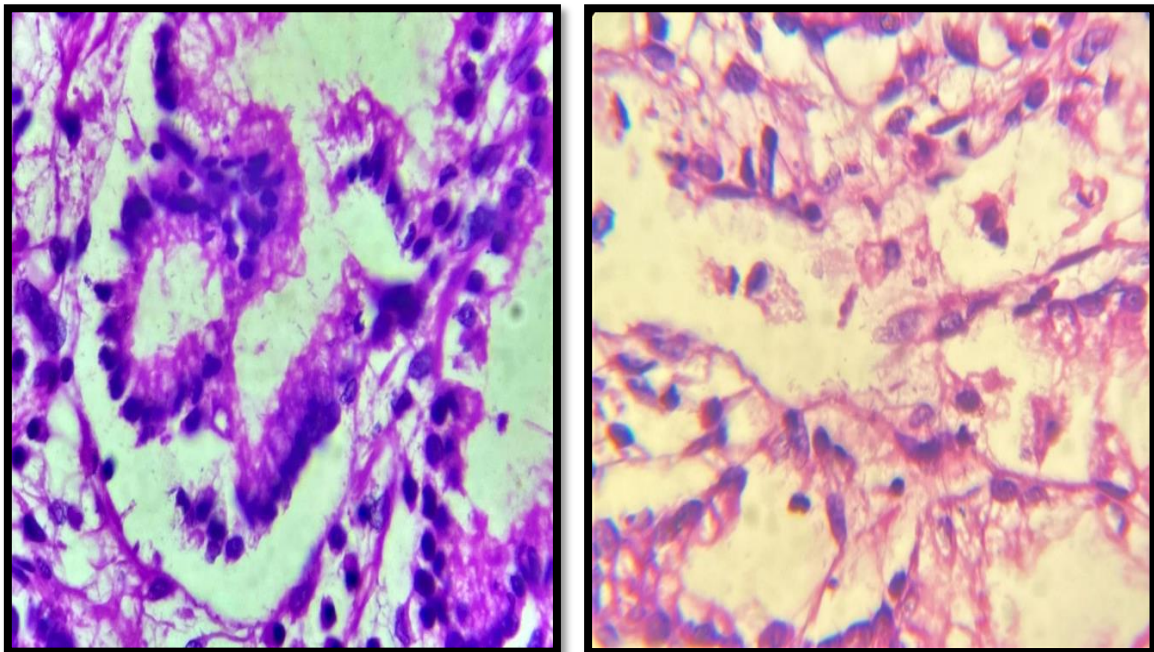
Comparison sensitivity, specificity, positive predictive value, negative predictive value of various histological stains.

Sensitivity — H & E: 51.85%, Warthin starry: 100%, Giemsa: 66.60%
Specificity — H & E: 95.65%, Warthin starry: 100%, Giemsa: 95.60%
PPV — H & E: 93.33%, Warthin starry: 100%, Giemsa: 94.70%
NPV — H & E: 62.85%, Warthin starry: 100%, Giemsa: 70.96%

Legend: ■ H & E ■ Warthin starry ■ Giemsa

GRAPH: 4

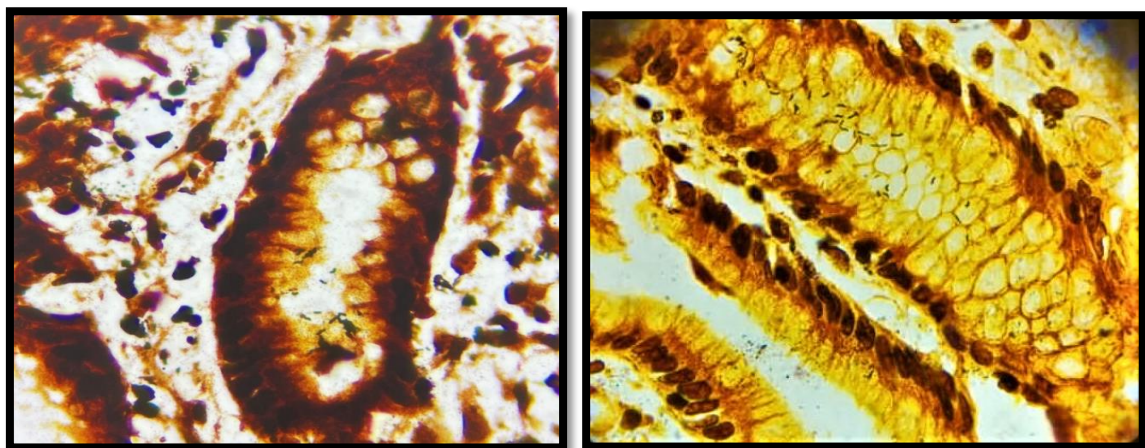**Figure 1 and 2**: Few pinkish spiral curved organism in the glands (100x) (H &E).

Figure 3 and 4 : Numerous black curved organisms against brown contrast of surrounding tissue in luminal aspect of the gland(100x) (Warthin starry stain)
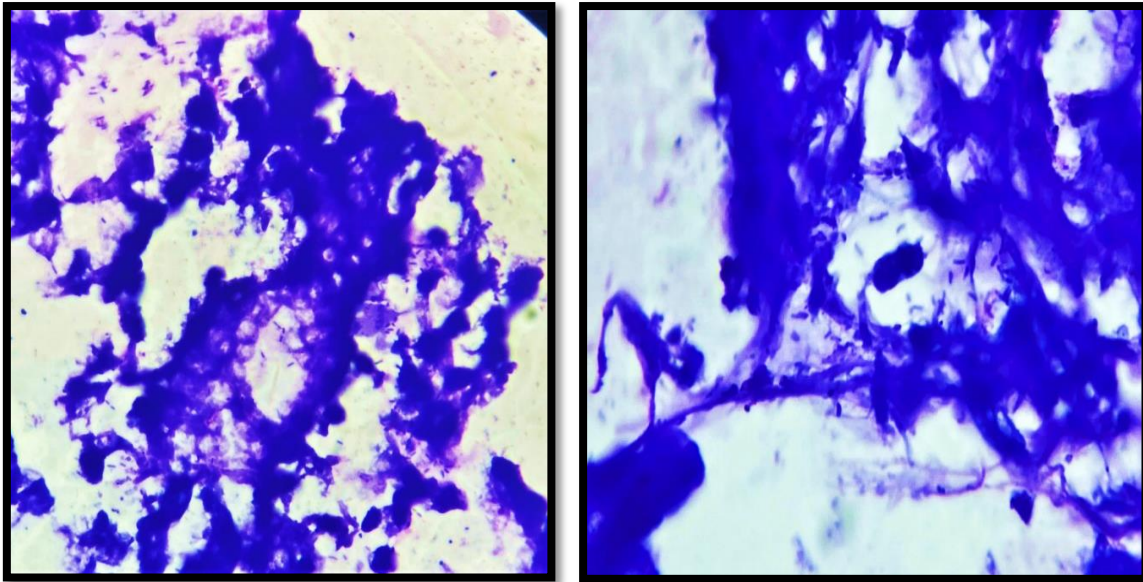
Figure 5 and 6: Blue curved organism in luminal aspect of the gastric glands (40x) (Giemsa Stain)

Discussion:

Since the astonishing discovery of H. pylori by Marshall and Warren launched revolutions in medical field, leading to development of various diagnostic methods for the aim of accurate diagnosis of this organism. Nearly half of the population of world is infected with H. pylori and most of them belong to developing countries.[6] An Endoscopy is a standard first line investigation for patients with GI symptoms and in combination with adequate endoscopic biopsy for histological examination, provides precise diagnosis of the lesion and monitoring the course of a various gastro-intestinal lesions.[7]

Histological examination is a method of choice for identification of H. pylori infection. There are several staining methods for detection of H. pylori and various studies have been done and have emphasized that staining with hematoxylin and eosin might miss the bacteria due to lack of contrast between the organism and the surrounding gastric mucosa or because of bacterial contaminants. Special staining with silver impregnation Warthin-starry stain is considered gold standard along with Giemsa stain for the detection of H. pylori.[8]

The present study showed an overall incidence of H. Pylori in 27/50 cases (54%) which is comparable with the studies done by Kumar V et al.,[9] (51%) followed by P. Sharma et al.,[10] (47%) and S. Adlekha et al.,[11] (62%) respectively.

On correlation of endoscopic and histopathology diagnosis in our study, endoscopic findings of 27 H. pylori positive cases were compared with histopathological findings. Out of which, 15 cases (56%) of gastritis showed concordance on histopathological diagnosis, similar findings were seen in the study which was done by Chattopadhyay G.[12]

Our study showed 54% of positive H. pylori cases which were confirmed by different staining methods as routine H&E (28%), Warthin-Starry (54%), and Giemsa (38%) respectively. Our study was comparable with other studies. Smita S Sonawane et al.,[13], Sandhika [14] and M. Ashton Key et al.,[15] found 55%, 63.33% & 66% positivity with Warthin-Starry stain respectively. Fiaz Ahmad et al.,[16], M. Ashton Key et al.,[15] and RaziyeTajalli et al.,[17] found 34%, 37% and 41.86% H. pylori positivity with H&E stain. Smita S sonawane et al.,[13] and Sandhika[14] found 53% and 53.33% H. pylori positivity with Giemsa stain.

In the present study we found Warthin starry staining method as gold standard showing 100% sensitivity, specificity, PPV and NPV respectively and was comparable with the study done by Himani B. Pandya et al.,[18] showing specificity (100%), sensitivity (84.5%), PPV (59.1 %) and NPV (100%). Another study by Cpt. Elizabeth A. et al.,[19] found sensitivity of (67%) and specificity of (100%).

The sensitivity, specificity, PPV, NPV of H&E stain in present study was (95.65%), (51.85%), (93.33%), (62.85%) respectively. Similar findings were seen in other studies done by Loren Laine et al.,[20], F.Kacar et al.,[21], Jen-En Tzeng et al.,[22] showing sensitivity as (92%), (97%) and (98.5%).

The specificity of H&E stain was less when compared with Warthin-Starry, although time consuming but the staining contrast was maximum. PPV of H&E stain was comparable with study done by Jen-En Tzeng et al.,[22]as (100%).
The sensitivity of 66.6% reported inGiemsa was correlated with RaziyeTajalli et al.,[17](53.49%). Specificity in our study was (95.6%), which was comparable to other studies,Himani B. Pandya et al.,[18]and RaziyaTajalli et al.,[17]. The lowspecificitydue

to low stain contrast and possibility of low density of H.pylori organism. However, our study showed unexpectedly low reliability of Giemsa staining with regards to history of medication, where the spiral form of the organism gets transformed to coccoid form which may not have been detected by this staining method as stated by RaziyeTajalli et al.,[17]. PPV of 94.7% was similar with the study of Jen-En Tzeng et al.,[22] (98.5%). NPV in present study withGiemsa was (70.96%) and was comparable with RaziyeTajalli et al.,[17](66.60%).

Conclusion:
The routine H and E stain supplemented with special stains like Giemsa and Warthin-Starry helps in identification of the spiral organism Helicobacter Pylori, thereby helps in giving prompt treatment and prevention of its sequalae. This study was also helpful in diagnosing various gastric pathologies caused by H. pylori.

The different staining methods confirmed the positivity of H. pylori in 27 cases, showing positivity of Warthin Starry (54%), Giemsa (38%) and H and E (28%).

The specificity, sensitivity, PPV, NPV of special stains with Warthin-Starry was 100% each, H&E was 51.85%, 95.65%, 93.33% and 62.85%, modified Giemsa was 95.6%, 66.6%, 94.7%, 70.96% respectively.

The present study highlighted the importance of incidence, endoscopic biopsies, identification of H. pylori and various gastric pathologies. The study also revealed the usefulness of different staining methodologies like H and E, modified Giemsa and Warthin-Starry stain in the identification of H. pylori in various gastric pathologies. H and E and Modified Giemsa staining techniques were found to be simple, cost effective and less time consuming than Warthin starry staining which is costlier and time consuming yet proved to be the gold standard for diagnosing H. pylori due to its high sensitivity, specificity and good staining color contrast. Hence, chances of missing the organism are least with Warthin-Starry stain.

References:
1. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum*. 1994;61:1-241.
2. Ansari, S., Gautam, R., Nepal, H.P. *et al. Helicobacter pylori* colonization in Nepal; assessment of prevalence and potential risk factors in a hospital-based patient cohort. *BMC Res Notes* **9,** 59 (2016).
3. Rotimi O, Cairn A, Gray S, Moayyedi P, Dixon M. Histological identification of Helicobacter pylori: Comparison of staining methods. J Clin Pathol. 2000 Oct: 53(10): 756- 759.
4. Shukla S, Pujani M, Agarwal A, Pujani M, Rohtagi A. Correlation of Serology with Morphological Changes in Gastric Biopsy in Helicobacter Pylori Infection and Evaluation of Immunohistochemistry for H. Pylori Identification. Saudi J Gastroenterol. 2012 Nov-Dec; 18(6): 369–374. doi: 10.4103/1319- 3767.103428.
5. Hussain, S.I., Reshi, R., Akhter, G., &Beigh, A. (2015). Clinico histopathological study of upper gastrointestinal tract endoscopic biopsies. *International journal of current research and review, 7*, 78-85.
6. Dr.Smita S. Sonawane, Dr. Milind V. Patil, and Dr.Kalgutkar, A.D., 2015. "Histopathological grading and Comparison of staining methods for H Pylori detection" International Journal of Current Research, 7, (11), 23043-23048.
7. Sharma S, Makaju R, Dhakal R, et al. Correlation between Endoscopic and Histopathological Findings in Gastric Lesions. Kathmandu University Medical Journal (KUMJ). 2015 Jul-Sep;13(51):216-219. DOI: 10.3126/kumj.v13i3.16808. PMID: 27180366.
8. Sharma R P, Kumari MK K. Histological Identification of Helicobacter pylori: Comparison of staining methods. Int J Med Res Rev [Internet]. 2015Nov.30 [cited 2021Nov.14];3(10):1167-73.
9. Akanda MR,1 Rahman AN2 Comparative Study of Different Methods for Detection of Helicobacter Pylori in Gastric Biopsies [Dinajpur Med Col J 2011 Jan; 4 (1):1-6].
10. Robert M. Genta, MD, George o. Robason, HT (ASCP), and David Y. Graham, MD. Simultaneous visualization of Helicobacter pylori and gastric morphology: A new stain. Human Pathology, Volume -25, No.3 (March 1994); 221-226.
11. S. Adlekha, T Chadha, P Krishnan and B Sumangala. Prevalence of Helicobacter pylori infection among patients undergoing upper gastrointestinal endoscopy in a medical college hospital in Kerala, India. Ann Med Health Sci Res. 2013 Oct-Dec; 3(4):559-563.
12. Chattopadhyay G', Basu K, Mukherjee S, Hazra BR. Gastroduodenal mucosa in peptic ulcer: endoscopic and histological assessment. Trop Gastroenterol. 1997 Dee; 18(4):156-9.
13. Dr.Smita S. Sonawane, Dr. Milind V. Patil, and Dr.Kalgutkar, A.D., 2015. "Histopathological grading and Comparison of staining methods for H Pylori detection" International Journal of Current Research, 7, (11), 23043-23048.
14. Sandhika, W. (2019). DETECTION OF HELICOBACTER PYLORI INFECTION IN CHRONIC GASTRITIS BIOPSY SPECIMEN USING WARTHIN-STARRY AND 95 MODIFIED GIEMSA STAIN IN DR SOETOMO HOSPITAL SURABAYA. Indonesian Journal of Tropical and Infectious Disease, 7(6), 150-154. doi:http://dx.doi.org/10.20473/ijtid.v7i6.8404
15. M Ashton-Key, T C Diss, P G Isaacson. Detection of helicobacter pylori in gastric biopsy and resection specimens.J clin pathol 1996; 49: 107-111.
16. Faiz Ahmad, Rozina Jaffar, Inamullah Khan. Helicobacter pylori detection in chronic gastritis: A comparison of staining methods. J Ayub Med ColI Abbottabad 2011; 23(2).

17.   Raziye Tajalli,1,2 MalihehNobakht. Hajar Mohammadi-Barzelighi,' Shahram Agah, AbdolazizRastegar-Lari, and Alireza Sadeghipour' . The Immunohistochemistry and Toluidine Blue Roles for Helicobacter pylori Detection in Patients with Gastritis. Iran Biomed J. Jan 2013; 17(1): 36-41.
18.   Himani B. Pandya. Jagdish S. Patel, Harihar H. Agrawat, Sahil B. Patel, Minai C. Thakkar. Identification of Helicobacter pylori by different conventional staining techniques and its comparison with polymerase chain reaction. Saudi Med J 2013; Vol. 34 (9): 942-948.
19.   Cpt. Elizabeth A. Motgomery, M.D., Maj. David F. Martin, M.D., And Col. David A. Peura, M.D. Rapid diagnosis of campylobacter pylori by Grams stain. A.J.e.p. November 1988; 606-609.
20.   Loren Laine MD, David N.Lewin, Md,WesleyNaritoku, MD, Hartely Cohen, MD. Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori. Gastrointestinal Endoscopy 1997, volume 45, No.6. 98.
21.   F Kacar, N Culhac, V Yukselen, Meteo Lu, E Dikicio Lu, E. Levi. Histologic demonstration of Helicobacter Pylori in Gastric Biopsies: which is the best staining method? The internet journal of pathology. 2003: Volume 3 Number 1.
22.   Jeh-En Tzeng, Ying-Lung Lin1, Sue-Mei Chung, Yi-Tsui Chu. Comparison of Four Diagnostic Methods for Helicobacter pylori (Tzu Chi Med J 2005; 17:339-343) Claudio Doglioni, Michela Turrin, Ettore Macri, Concetta Chiarelli, BastianelloGermana, Mattia Barbereschi. J clin pathol1997; 50: 461-464.