Proceedings S.Z.M.C. Vol: 34(4): pp. 4-9, 2020. PSZMC-763-34-4-2020

Detection of Helicobacter pylori Through Histochemistry & Immunofluorescent Staining in Biopsies of Patients with Chronic Gastritis

Asia Jahanzeb, Abdul Hannan Nagi
Department of Morbid Anatomy & Histopathology, University of Health Sciences, Lahore

ABSTRACT

Introduction: Helicobacter pylori (H.pylori*)* is a helix shaped gram negative rod which is usually associated with chronic gastritis and also a major cause of other gastroduodenal diseases as well. **Aims & Objectives:** The present study used histochemical and immunofluorescent stains on formalin fixed paraffin embedded human gastric biopsies for detection of H.pylori. Comparison was also done to evaluate best staining method. **Place and duration of study:** This study was accomplished in about one year. Sampling of gastric biopsies and rapid urease test were executed at the endoscopy suite of Lahore General Hospital whereas the histopathological examination and immunofluorescent staining were done in University of Health Sciences, Lahore. **Material & Methods:** Thirty patients (n=30cases) were included in the study following inclusion criteria. Diagnostic upper GI endoscopy was carried out in all cases. Five gastric biopsies were taken from each patient/case (total N=150 Biopsies) according to the Updated Sydney System. Rapid urease test was performed at the site of endoscopy. Biopsies fixed in 10% formalin were brought to the concerned department where they were assigned a specific laboratory number then processed and stained. **Results:** Endoscopic examination revealed chronic gastritis and rapid urease tests were positive. All cases (n=30) were positive for H.pylori on histopathology. The calculated sensitivity and specificity of H&E, Giemsa, Modified McMullen's stain and Immunofluorescent method in present study were 71% and 100%,83% and 100%, 82% and 100%, 90% and 100% respectively. **Conclusion:** Special stains makes H.pylori identification easier in tissue sections. However, immunofluorescent test is the most sensitive and specific method as compared to histochemical stains.

Key words: Helicobacter pylori, chronic gastritis, Rapid urease test, Immunofluorescent stain.

INTRODUCTION

In 1982, H.pylori was first discovered by Drs. Barry Marshall and Robin Warrens in gastric mucosa of patients having gastritis and ulcers.[1] H.pylori causes Chronic Gastritis and has been related with other gastroduodenal diseases including peptic ulcer diseases (PUD), gastric and duodenal ulcers, mucosa associated lymphoid tissue (MALT) lymphoma and even gastric carcinoma.[2] More than 50% of patients reveals H.pylori upon endoscopy as humans are the principle host and it predominantly inhabits human gastric mucosa that leads to inflammation and other pathological change.[3] Numerous studies have reported different incidence rates of H.pylori infection that varies broadly due to topographical area, age, race, culture and living standards. Infections are more common in developing countries due to poor living and sanitary conditions.[4] So penurious living conditions, poor hygiene, overpopulation and genetic susceptibility are the possible risk factors of H.pylori infection. Presence of H.pylori DNA in saliva, vomitus, gastric juices, feces and water shows its diverse transmission sources.[4] Presence of virulence genes of H.pylori in different samples of drinking water is also reported from Pakistan.[5]

In Pakistan, the prevalence of H.p*ylori* infection is also high like other developing countries and reported in the range of 50–90%.[6]

Several H.pylori virulence factors like enzymes, toxins and genetic factors are known that are involved in the pathogenesis of diseases.[7] Cytotoxin associated gene (CagA**)** of H.pylori is potentially considered as a carcinogen.[8] Virulent H.pylori strains elicits more powerful inflammatory response and are related with increased risk of gastric carcinoma.[9]

Various invasive and non-invasive tests are available for H.pylori diagnosis.[10] Endoscopy is the most frequently practiced invasive procedure today and histological examination provides additional information ranging from inflammatory to malignant conditions.[11] So, histochemical stains and immunofluorescent (anti H.pylori) method were used in study for detection of Helicobacter pylori. Statistical analysis was done to evaluate the most sensitive and specific method.

MATERIAL AND METHODS

After approval by Ethical Review Committee UHS/Education letter no. 126-17/863**.** This diagnostic study was conducted in UHS during period from June 2017 to July 2018.

Convenient sampling technique was used. Data, history and gastric biopsy of adult dyspeptic patients admitted to Lahore General Hospital were taken after obtaining written consent.

Exclusion criteria: Patients with co-morbid conditions (e.g. Hepatitis B, C), patients undergoing antibiotic or proton pump inhibitor (PPI) treatment were excluded**.**

Inclusion criteria: Thirty patients (n=30) of both gender and age range of 18-45 years reporting for the primary diagnosis of the disease were enrolled in present study.

Endoscopy: It was done in Lahore General Hospital by gastroenterologist using Olympus CV-190 video endoscope. Five biopsies (two each from antrum and corpus and one from incisura angularis) were taken from 30 patients (total N=150 biopsies).[11] In present study two biopsies (one each from antral and corpus (greater curvature) were used for the rapid urease test in each patient and other biopsies were fixed in 10% formalin solution for histopathological examination. The biopsy sections were stained with H&E, Giemsa and Modified McMullen's stain.[12,13]

Grading of gastritis was done following updated Sydney Classification. Histopathological variables like activity (neutrophil infiltration), chronic inflammation (mononuclear cell infiltration), atrophy (loss of normal glands from antrum or corpus) and Gastric intestinal metaplasia (replacement of gastric epithelium by intestinal type) were also documented that also defined the prognosis of gastric infection in study population.[14]

Immunofluorescent staining: Polyclonal anti H.*pylori* antibody conjugated with FITC (Fluorescein iso-thiocyanate conjugate) was used on formalin fixed paraffin embedded gastric biopsies.[15] Fluorescence microscope (OLYMPUS BX51), Model U-LH1OOHG was used for microscopy and images were captured. FITC usually gives Apple green fluorescence that locates the antigen in tissue.[16]

Statistical analysis:

The data was analyzed using SPSS (20.0). Mean and standard deviation were considered for quantitative variables in addition to the frequencies along with percentages for qualitative variables. Categorical variables were investigated by using Pearson's chi-square test. A p-value less than 0.05 ($p<\_0.05$) was taken significant. Comparison of staining methods was done and statistical sensitivity, specificity, Negative predictive value, positive predictive value and diagnostic accuracy were also calculated.[17]

RESULTS

Mean age of the population was 34±8. The gender distribution showed that females were predominant that is n=17 (56.7%) and n=13 (43.3%) were males. The female to male ratio was 1.3:1. The mean age of males and females were 29.92±7.6 and 36.12±7.8 years respectively. Regarding localization and distribution of gastritis, the most common gastritis on histopathology was Antral gastritis n=16 (53.3%), antral predominant pan gastritis and corpus predominant pan gastritis was seen in n=11 (36.7%) and n=3 (10%) of the cases respectively.(Table 1)

Clinic endoscopic features	**n**	**%age**
Age		
18-30years	9	30%
31-45 years	21	70%
Hyperemia	26	86.7%
Erosion	1	3.3%
Ulceration	1	3.3%
Nodularity	0	0
Co-exiting duodenitis	2	6.7%
Location of endoscopic gastritis		
-Antral gastritis	16	53.3%
-Corpus predominant	3	10%
-Antral predom Pangastritis	11	36.7%
Normal Gastric Mucosa	0	0%

Table-1: Distribution of cases with respect to clinical and endoscopic data

Morphological Variables	G0 (Nil)	Grades G1 (Mild)	G2 (Moderate)	G3 (marked)
H. pylori Density	n = 0 none	n = 4 1-3 bacteria	n=11 layer of bacteria	n=15n clusters
Activity (neutrophil infiltrate)	n =18 60%	n = 12 40%	n=o	n=0
Chronic inflammation (lymphocytic infiltrate)	n = 0	n = 4 13.3%	n=11 37%	n=15 50%
Glandular atrophy	n=27 (90%)	n=0	n=1	n=2 6.6%
Gastric intestinal metaplasia	n=30 (100%)	n=0	n=0	n=0

Table-2: Grading of histopathological variables of antral and corporal mucosa according to the Updated Sydney System.

Go=No inflammation, G1=mild inflammation, G2=moderate inflammation, G3 marked inflammation. H.pylori density = mild, moderate and marked number of H.pylori overlying epithelium.

Density was observed in all n=30 cases of chronic gastritis. Cases of G1 and G2 grades of chronic gastritis showed low density of bacteria while n=15 cases of G3 showed high density. G3 i.e. marked chronic gastritis was the most commonly observed grade of chronic inflammation. The grade of activity was mild for n=12 (40%) cases. However no activity was observed in n=18 (60%) of the cases. The finding of chronic inflammation was observed in all n=30 (100%) cases of which n=15 (50%) cases had marked chronic inflammation, n=11 (36.7%) cases had moderate chronic inflammation and n=4 (13.3%) cases had mild chronic inflammation. Histologically n=2 (6.6%) cases of marked chronic inflammation revealed atrophy but no gastric intestinal metaplasia was observed (Table-2).

Staining	Sensitivity A/A+C x100	Specificity D/B+Dx 100	PPV A/A+B x100	NPV D/D+C x100	Accuracy A+D/A+B +C+Dx100
H&E	71%	80 %	98	20	66%
Giemsa	83%	100%	100	14.2	84%
Modified McMullen's	82%	100%	100	16.6	83%
Immunofluorescent	90%	100%	100	0.00	90%

Table-3: Statistical analysis

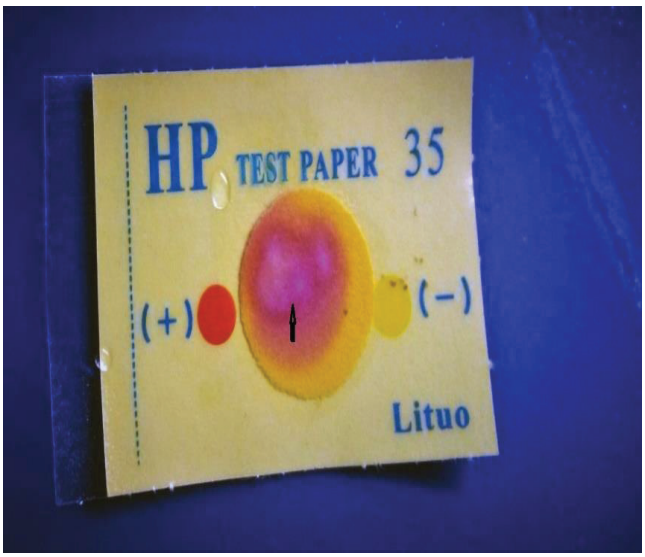

Fig-1: Rapid Urease Test, strong positive result, Test paper turns from yellow to cherry red

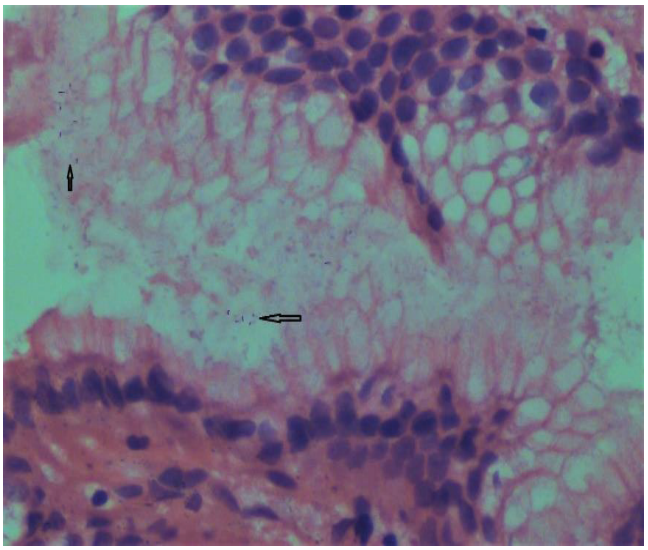

Fig-2: H&E shows H.pylori in gastric pits

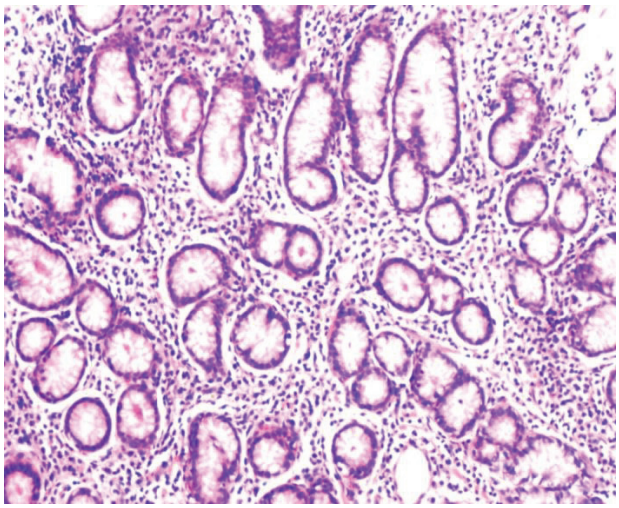

Fig-3: H&E Chronic mild antral gastritis

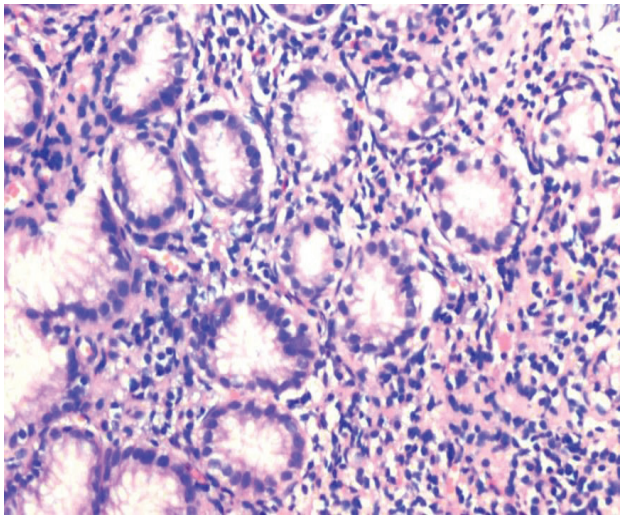

Fig-4: H&E Chronic Moderate antral Gastritis

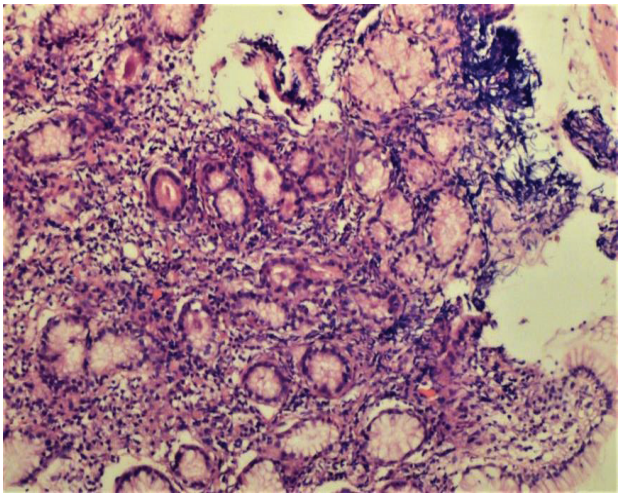

Fig-5: H&E Chronic marked antral gastritis

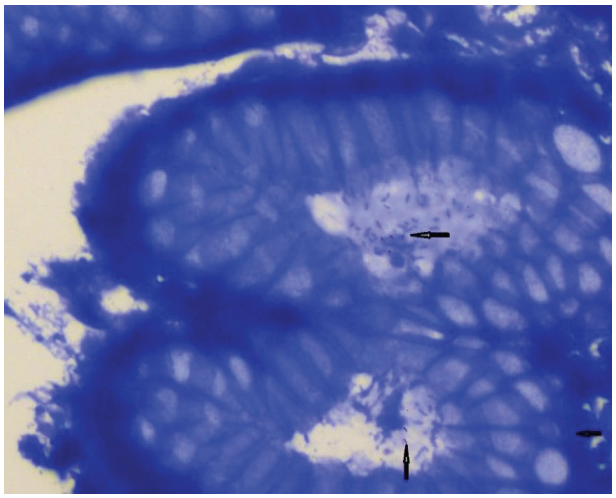

Fig-6: H.pylori on Giemsa staining 40X

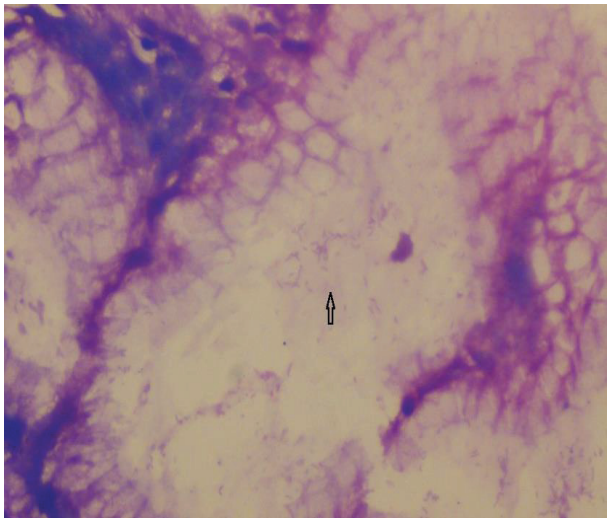

Fig-7: H.pylori in gastric pits on Modified McMullen's Stain 40x

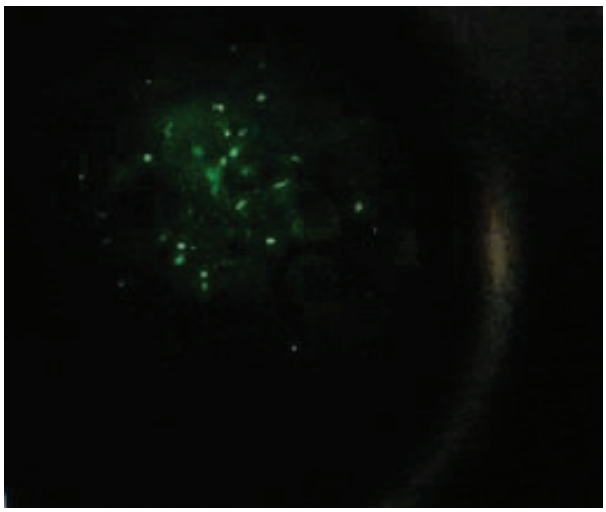

Fig-8: Immunofluorescent stain (Apple green fluorescence)

DISCUSSION

H.pylori induced gastritis is the most common gastric infection that fluctuates in diverse areas of world depending on several factors that effects its acquisition, transmission and pathogenesis. The prevalence in our study came out to be 100% that was relatively higher as compared to the previous study reported.[6] This was probably due to small sample size of our study in which we had included patients whose severe clinical symptoms were suggestive of chronic gastritis. Females were predominant in present study that was contrary to previous studies that showed the predominance of males. This can be related to low educational status of females in Pakistan and increased exposure to risk factors like domestic hygiene, large number of siblings with crowded living conditions and poor sanitation. Zoonotic and vector borne transmission

could be a source but considered the lowest risk factor.[4,6]

The mean age in our study population was 34±8.4 that was less compared to previous studies.[18] This was due the inclusion criteria of our study i.e. the age range was 18-45 years and patients under 18 years and above 50 years of age were excluded. However, the relation of infection with age cannot be explained and justified in present study because pediatric and elderly patients were not incorporated in this study.

The clinical symptoms presented by patients were in accordance to the previous studies reported both locally and internationally.[19] Rapid urease test was performed immediately after endoscopy using commercially available kits (Lituo Biotech) and observed to be positive in all cases n=30 (100%). These findings were consistent with both local and international studies.[20] Cases were also evaluated on basis of yield of H.pylori at various anatomical sites and it was found that probability of finding H.pylori was highest at the antrum.

In present study G3 i.e. marked chronic gastritis was the most commonly observed grade of chronic inflammation probably because patients had the symptoms of gastritis since 8-9 years. Activity (active inflammation) was seen in n=12 (40%) cases, a finding which is accordant to both local and international studies.[6] Gastric intestinal metaplasia (GIM) cases were not found in present study that represented very low risk of developing gastric carcinoma in our study population due to small sample size of our study but a large number of population would be needed to assess the risk of gastric cancer.

The sensitivity and specificity of H&E staining were found to be 71% and 80 % respectively that were comparable to previous studies.[11] (Table3).

The sensitivity and specificity of Giemsa staining were ā83% and 100% respectively and compatible with the previous studies.[11] The sensitivity and specificity of modified McMullen's stain were 82% and 100 % respectively and compatible with previously done researches.[12,13]

Statistical analysis in present study showed that Immunofluorescent test was the most sensitive and specific test for H.pylori identification as the *P v*alue was less than 0.05,X2 (0.05)=1.16. (Table3) The calculated sensitivity and specificity of immunofluorescent test in our study were 90% and 100% respectively probably due to strongly positive cases of H.pylori, but compatible with previous study.[15]

The antibody (polyclonal anti H. pylori antibody) used in Immunofluorescent assay did not react with the closely matched bacteria present in gastric mucosa thus detected even low concentration of an antigen in tissue as in corpus biopsies where the yield of bacteria was usually low. In this study no false positive cases were revealed due to strongly positive cases of H.pylori induced chronic gastritis.

CONCLUSION

H.pylori associated chronic Gastritis is an appalling issue in our country which needs to be noticed seriously. Modified McMullen's stain can be used in pathological laboratories like Giemsa stain for detection of H.pylori due to its better contrast. Immunofluorescent staining is the most sensitive and specific method but it is not being used in pathological laboratories commonly due to its high cost and complexity. It should be restricted to research based studies. Every pathological laboratory desires to use cheap and simple method in routine practice with much emphasis on time/ labor cost. So, Giemsa stain is still the most economical and convenient staining method that would help as good as immunofluorescent method in our setting where resources are limited.

Limitations of the study:

Disposable biopsy forceps, which is not routinely practiced in our hospitals, were expensive. Immunofluorescent assay was also an expensive and time consuming procedure that's why due to budget and time we were restricted to smaller sample size. So, a project on higher scale would be more persuasive and statistically important to access the efficacy of endoscopic biopsies, histochemical stains and immunofluorescent assay as well as its diagnostic and prognostic role in gastric infections.

Acknowledgement:

We are thankful to Dr. Nadia Naseem, Head Department of Morbid Anatomy and Histopathology, UHS Lahore.

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet (London, England). 1984; 1(8390):1311-5.
2. Konturek JW. Discovery by Jaworski of Helicobacter pylori and its pathogenetic role in peptic ulcer, gastritis and gastric cancer. Journal of Physiology and Pharmacology : an official journal of the Polish Physiological Society. 2003; 54 Suppl 3:23-41.

3. McNulty CA. Campylobacter pyloridis-associated gastritis. The Journal of infection. 1986; 13(2):107-13.
4. Brown LM. Helicobacter Pylori : Epidemiology and Routes of Transmission. Epidemiologic Reviews. 2000; 22(2):283-97.
5. Samra ZQ, Javaid U, Ghafoor S, Batool A, Dar N, Athar MA. PCR assay targeting virulence genes of Helicobacter pylori isolated from drinking water and clinical samples in Lahore metropolitan, Pakistan. Journal of water and health. 2011; 9(1):208-16.
6. Mehmood K, Awan AA, Muhammad N, Hasan F, Nadir A. Helicobacter pylori prevalence and histopathological findings in dyspeptic patients. Journal of Ayub Medical College, Abbottabad : JAMC. 2014; 26(2):182-5.
7. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clinical microbiology reviews.2006;19(3):449-90
8. Hatakeyama M. Helicobacter pylori CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. Cell host & microbe. 2014; 15(3):306-16.
9. Wroblewski LE, Peek RM, Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clinical microbiology reviews. 2010; 23(4):713-39.
10. Best LM, Takwoingi Y, Siddique S, Selladurai A, Gandhi A, Low B, et al. Non-invasive diagnostic tests for Helicobacter pylori infection. The Cochrane database of systematic reviews. 2018; 3:Cd012080.
11. Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Annals of translational medicine. 2015; 3(1):10.
12. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: comparison of staining methods. Journal of clinical pathology. 2000; 53(10):756-9.
13. Lwaki H, Sugiyama T, Asaka M. A modified McMullen's staining for Helicobacter pylori: a high-contrast, visibly prominent method. Helicobacter. 1998; 3(1):45-8.
14. Stolte M, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. Canadian journal of gastroenterology = Journal canadien de gastroenterologie. 2001; 15(9):591-8.
15. Rivera E, López-Vidal Y, Luqueño V, Ruiz-Palacios GM. Indirect immunofluorescence assay for detection of Helicobacter pylori in human gastric mucosal biopsies. J Clin Microbiol. 1991; 29(8):1748-51.
16. Makki JS. Diagnostic Implication and Clinical Relevance of Ancillary Techniques in Clinical Pathology Practice. Clin Med Insights Pathol. 2016; 9:5-11.
17. Baratloo A, Hosseini M, Negida A, El Ashal G. Part 1: Simple Definition and Calculation of Accuracy, Sensitivity and Specificity. Emerg (Tehran). 2015; 3(2):48-9.
18. Yakoob J, Abbas Z, Jafri W, Usman MW, Jafri F, Awan S. Comparison of the virulence markers of Helicobacter pylori and their associated diseases in patients from Pakistan and Afghanistan. Saudi J Gastroenterol. 2013; 19(5):211-8.
19. Suzuki H, Moayyedi P. Helicobacter pylori infection in functional dyspepsia. Nature reviews Gastroenterology & hepatology. 2013; 10(3):168-74.
20. Uotani T, Graham DY. Diagnosis of Helicobacter pylori using the rapid urease test. Annals of translational medicine. 2015; 3(1):9.

The Authors:
Asia Jahanzeb
Dept. of Morbid Anatomy & Histopathology,
University of Health Sciences, Lahore.

Prof. Abdul Hannan Nagi
Former Head,
Dept. of Morbid Anatomy & Histopathology,
University of Health Sciences, Lahore.

Corresponding Author:
Asia Jahanzeb
Dept. of Morbid Anatomy & Histopathology,
University of Health Sciences, Lahore.
E-mail: asaiarao1000@gmail.com