DETECTION OF H. PYLORI IN GASTRIC BIOPSIES: A COST EFFECTIVE METHOD FOR DEVELOPING COUNTRIES.

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ABSTRACT… H. pylori is the most common organism implicated in the pathogenesis of chronic gastritis. The aim of this study is to assess the reliability and cost effectiveness of hematoxylin and eosin (H&E) stain for the detection of H. pylori in gastric biopsies for developing countries with immunohistochemical markers (IHC) as gold standard. **Study Design:** Descriptive cross-sectional study. **Setting:** OPD of Kuwait teaching hospital and Mercy teaching hospital. **Period:** April 2017 to December 2017. **Materials and Methods:** 50 consecutive endoscopic gastric biopsies collected according to the Updated Sydney Protocol from patients with dyspeptic symptoms. The data was entered and analyzed in SPSS version 19. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each staining technique. Chi square test was used for calculating p value. A value less than or equal to 0.05 was considered statistically significant. **Results:** The most common presenting symptom was epigastric pain, nausea/vomiting and bloating/burping. According to Updated Sydney Protocol, the most common diagnosis was mild chronic inflammation. Sensitivity, specificity, p ositive predictive and negative predictive value of H&E was 46.8 %, 88.8%, 88.2% and 48.5% respectively. **Conclusion:** Our study concludes that used alone H&E can easily detect H. pylori in gastric biopsies in most of the cases. However, IHC may be used in selected patients with negative H&E results and strong clinical suspicion of H. pylori infection.

INTRODUCTION

Chronic gastritis has remained an underrated disease despite millions of deaths worldwide occurring from its sequel like peptic ulcer and gastric cancer.[1] Researches on pathogenesis of gastritis gained popularity after the discovery of bacterium Helicobacter pylori (H. pylori) by Marshall and Warren in 1982 which resulted in a paradigm shift in the understanding of gastric diseases.[2]

H. pylori are small, highly motile, microaerophilic, gram negative, campylobacter like organisms, well known to be the most common human infection worldwide.[3] Chronic gastritis is prevalent in approximately 50 % of world population.[4] In Pakistan the frequency of H. pylori was found to be 88.3%.[5]

H. pylori is involved in the etiology of peptic ulcer, gastric adenocarcinoma and Mucosa Associated Lymphoid Tissue Lymphoma (MALTOMA).[6,7] WHO labeled H. pylori as a Class 1 carcinogen in 1994, which gave a whole new perspective to the understanding of gastric carcinogenesis.

Although routine H&E stained sections are sufficient to detect H. pylori[8] but in cases of atrophic mucosa and post eradication therapy ancillary stains may be required due to low bacterial count.[9] IHC technique uses the principle of antigen binding to specific antibodies in tissue. Antigen-antibody complexes are visualized by conjugation of peroxidase enzyme to the antibody and by tagging the antibody to a fluorophore (rhodamine, fluorescein). According to Ju Yup Lee et al H&E and Giemsa are adequate for an accurate diagnosis of H. pylori[9] but Razaiye T et al quoted that IHC has been found to be superior to other histochemical stains in false negative H.

pylori gastritis.[10]

The aim of this study is to assess the reliability and cost effectiveness of H&E for developing countries and compare it with IHC as gold standard for the detection of H. pylori in gastric biopsies.

MATERIALS AND METHODS
This descriptive cross-sectional study included endoscopic gastric biopsies of 50 consecutive patients with dyspeptic symptoms who presented to OPD of Kuwait Teaching Hospital and Mercy Teaching Hospital, Peshawar from April 2017 to December 2017. Patient's profile, signs/symptoms, history of NSAID and Proton Pump Inhibitors (PPI) intake were noted on a predesigned proforma. The study was approved by the Ethical Review Board of Peshawar Medical College.

Biopsy punches were taken according to the Updated Sydney Protocol (USP), with two biopsy punches from Antrum and Corpus each and one punch from incisura angularis. Biopsies were fixed in 10 % Buffered formalin and sent to Histopathology Laboratory, Peshawar Medical College. Patients with incomplete information and biopsies with autolytic changes were excluded due to antigen retrieval problem. Gastric biopsies were routinely processed. Two slides were kept for immunohistochemistry and one as reserve. Slides were observed on 100X power to enhance the probability of finding H. pylori. The histological features were recorded according to USP.

Immunohistochemistry was performed at Histopathology Diagnostic Laboratory, Peshawar Medical College. Peshawar, using Polyclonal Rabbit Anti-Helicobacter Pylori antibody. Hot air oven method was used for Antigen retrieval. Controls were used for comparison while assessing the slides.

The data was entered and analyzed in SPSS version 19. For continuous variables mean was calculated. For categorical variables frequency and percentages were calculated. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for H&E. Chi square test was used for calculating p value. A value less than or equal to 0.05 was considered statistically significant.

RESULTS
A total of 50 patients with dyspeptic symptoms undergoing endoscopic biopsy were included in this study. Among these 50 cases of gastric biopsies 24 (48%) were males and 26 (52%) were females. Male to female ratio was 1:1.1 (Table-I). The present study showed majority of females (61.1%) being H. pylori positive in comparison with males (53.1%).

The age ranged from 11-70 years with a mean of 40 years. Maximum number of H. pylori positivity was seen in the extreme of ages, 100% in the age group 61-80 (n=2/2) years and 66% (n=2/3) in the age group 01-20 years. It was followed by 65% (n=13/20) in the age group 41-60 years and 60% (n=15/25) in the age group 21-40 years (Table-II).

The most common presenting symptom was epigastric pain, nausea/vomiting and bloating/burping. Amongst the 45 patients with epigastric pain 28 (62.2%) were positive for H. pylori by IHC and 17(34%) by H&E. The least common symptom was hematemesis and melena were present in 5 of patients, 4 (80%) of which were positive for H. pylori by IHC and 2 (40%) by H&E (Table-III). No significant association was found between a particular symptom and H. pylori positivity.

The cases were diagnosed according to the USP. Mild chronic inflammation was found in 25 (50%) cases, whereas moderate chronic inflammation in 22 (44%) and severe chronic inflammation in 3 (6%) of the cases (Table-IV).

According to the severity of inflammation, the staining ability of H&E and IHC showed that 13 out of 25 cases of Mild Chronic Inflammation were positive for H. pylori by IHC and only 4 by H&E with 9 cases as false negative. Among the 22 cases with Moderate Chronic Inflammation, 16 were H. pylori positive on IHC and 10 on H&E with 5 false negative cases. In the three severe chronic

inflammation cases, H. pylori was diagnosed by IHC in all 3 and only one case was missed by H&E (Table-V).

In gastric biopsies H&E was positive for H. pylori in 34% of cases and negative in 66%. Whereas IHC showed 64% cases of H. pylori positive and 36% as negative. Sensitivity of H&E was 46.8 % and specificity was 88.8%. Positive predictive value of H&E was 88.2% and negative predictive value was 48.5% (Table-VI).

Gender	H&E		IHC	
	Absent	Present	Absent	Present
Male	15	9	7	17
	(30.0%)	(18.0%)	(14%)	(34%)
Female	18	8	11	15
	(36.0%)	(16.0%)	(22%)	(30%)
Total	33	17	18	32
	(66.0%)	(34.0%)	(36%)	(64%)

Table-I. Gender distribution of H. pylori positive cases by IHC and H&E

Age in Years	H. pylori +ve n=32 (64%)	H. pylori –ve n=18 (36%)	Total	Percentage
01-20	2	1	03	66
21-40	15	10	25	60
41-60	13	7	20	65
61-80	2	0	02	100

Table-II. Comparison of age in H. pylori IHC positive and negative cases

Signs and Symptoms		H. Pylori on H&E		H. Pylori on IHC	
		Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)
Epigastric Pain	Present	28(56)	17(34)	17 (37.8)	28 (62.2)
	Absent	5(10)	0(0)	1 (20)	4 (80)
Nausea/Vomiting	Present	22(44)	11(22)	12 (36.4)	21 (63.6)
	Absent	11(22)	6(12)	6 (35.3)	11 (64.7)
Hematemesis/Melena	Present	3(60)	2(40)	1 (20)	4 (80)
	Absent	30(60)	15(30)	17 (37.8)	28 (62.2)
Bloating/Burping	Present	8(16)	7(14)	4 (26.7)	11 (73.3)
	Absent	25(50)	10(20)	14 (40)	21 (60)
Dyspepsia	Present	23(46)	11(22)	11 (32.4)	23 (67.6)
	Absent	10(20)	6(12)	7 (43.8)	9 (56.3)

Table-III. Comparison of chronic gastritis signs and symptoms with H. pylori status by IHC and H&E

Chronic Inflammation	Mild	Moderate	Severe	Total
	25 (5%)	22 (44%)	3 (6%)	50 (100%)
Active	12 (48)	20 (90)	3 (100)	35 (70)
Atrophy	8 (32)	14 (63.6)	3 (100)	25 (50)
Intestinal Metaplasia	2 (8)	0 (0)	0 (0)	2 (4)

Table-IV. Histopathological findings according to USP

Chronic Inflammation	H&E		IHC	
	H. pylori -ve	H. pylori +ve	H. pylori -ve	H. pylori +ve
Mild	21	4	12	13
Moderate	11	11	6	16
Severe	1	2	0	3
Total n (%)	33 (66)	17 (34)	18 (36)	32 (64)

Table-V. Detection of H. pylori using H&E and IHC according to degree of inflammation

	IHC Positive	IHC Negative	Total
H&E Positive	15	2	17
H&E Negative	17	16	33
Total	32	18	50

Table-VI. Comparison of H. pylori detection by H&E and IHC

DISCUSSION

Analysis of patient's characteristics in our study reveals majority of female patients being H. pylori positive with a male to female ratio of 1:1.1. Interestingly, international studies reveal a male predilection for H. pylori infection.[12-14] The difference may be due to geographical variation, race, genetic diversity and socioeconomic status.[15,16]

According to present study, the mean age of chronic gastritis patients was 39.9 ± 12.9 years. Our findings are consistent with a local study by Mehmood et al., in 2010, with 176 sample size showing the mean age of 39.6 ± 15.2. However, a study by Awaad et al. in Egypt (2014), with sample size of 100 and a study by Lissa et al. in Brazil (2012), with 94 chronic gastritis patients, showed mean age of gastritis patients as 47 and 48 ± 16 years respectively.

In this study maximum number of H. pylori positivity was seen in extreme of ages which is in accordance with other international studies concluding that prevalence of H. pylori increases with age.[4,17] However, recent studies show a marked reduction in H. pylori infection in the elderly which can be explained by the popularity of eradication therapies.[18]

According to histological diagnosis the current study showed maximum number of cases (50%) as mild chronic inflammation, followed by moderate chronic inflammation (44%) and only 03 (6%) as severe chronic inflammation. This was consistent with a national study, where 57.3% patients had mild inflammation, 42.6% had moderate chronic inflammation and 4.4% had severe chronic inflammation.[5] However in contrast to our findings, a study by Marcela S.B. et al. showed more cases of moderate to severe chronic inflammation (31.5%) as compared to mild (25.9%).[21]

The ability of H&E in diagnosing H. pylori according to the degree of inflammation was assessed. Our study showed that H&E could not be relied upon in cases of mild inflammation because 69.2% cases were missed by it in this category. However, the ability of H&E to detect H. pylori increased with severity of inflammation which is in accordance with other studies.[7,22] In cases of mild inflammation IHC stain detected cases missed by H&E which is in agreement with a number of past studies.[21,23]

For a developing country like Pakistan, H&E fulfills the need of a cost-effective and efficient diagnostic tool for H. pylori diagnosis although less accurate than IHC[8,19], and in comparative analysis showed a low sensitivity and specificity.[24,25]

CONCLUSION

Our study concludes that used alone H&E can easily detect H. pylori in gastric biopsies in most of the cases. However, IHC may be used in selected patients with negative H&E results and strong clinical suspicion of H. pylori infection. **Copyright© 15 Mar, 2019.**

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2	Sadaf Alam	Critical analysis.	
3	Shahnawaz Khan	Review	
4	Mumtaz Khan	Final approval of the version to be published.	
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