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Original Article

Diagnostic Accuracy of Microbiological Culture for the Diagnosis of Helicobacter pylori Infection in Dyspeptic Patients taking Histopathological Examination as Gold Standard

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ABSTRACT

Objective: To accurately estimate the efficacy of microbiological culture technique in the diagnosis of H. pylori infection.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Gastroenterology, Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, from Jan to Sep 2021.
Methodology: Our study enrolled patients older than 18 years, who presented to PEMH during study period with history of chronic dyspepsia and underwent upper gastrointestinal endoscopy following which gastric tissue specimens were sent for histopathological and microbiological diagnosis. Cohen's kappa coefficient (κ) was determined to estimate the statistical correlation between the two diagnostic measures.
Results: A total of 73 patients having a chronic history of dyspepsia lasting approximately 7.0±4.0 months were studied. Mean patient age was 45.8±14.72 years, with 63% participants being males and 62% of participants had consumed Proton Pump Inhibitors (PPIs) for <12 weeks. A positive diagnosis for H. pylori infection was seen among patients with poor socioeconomic status and with a shorter duration of PPI therapy. Cohen's coefficient revealed only a moderate level of agreement between histopathological diagnosis and microbiological culture technique (κ=0.60). Furthermore, microbiological diagnosis had a drastically lower sensitivity (66.7%) and specificity (92.6%) with diagnostic accuracy of 78.1 % as compared to its counterpart.
Conclusion: Microbiological culture technique was found to have an inferior level of accuracy in the laboratory detection of H. pylori in contrast to histopathological diagnosis.

Keywords: Helicobacter pylori; Histopathology; Microbiology; Dyspepsia; Proton Pump Inhibitors.

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INTRODUCTION

Helicobacter pylori is a common microbiological pathogen inhabiting the gastrointestinal tract (GIT).[1], infecting up to half of the general population, making it a proven risk factor in the pathogenesis of peptic ulcer disease (PUD) and chronic gastritis as the risk of gastric atrophy increases following an infection with H. pylori, making patients highly susceptible to gastric carcinoma, however, eradication of H. pylori drastically reduces the overall risk.[2] This role in a multitude of GI-related complications necessitates an accurate and timely diagnosis.[3] for which several diagnostic modalities are used in clinical practice. Histopathological examination following gastric biopsy is the gold-standard in the diagnosis of H. pylori infection where gastric antrum is the most commonly selected point for specimen extraction.[4] Despite promising sensitivity (95%) and specificity (99%), histopathological testing can partially overlook GI colonization by H. pylori, secondary to continuous use of proton pump inhibitors (PPIs), dietary factors, inaccurate staining technique, or inappropriate selection of biopsy site.[5] Microbiological culture and sensitivity carries a significantly high specificity (~100%) but its sensitivity shows variability,[6] of 85-95% while some authors have noted a far lower value (~45%).[5,7]. H. pylori culture requires maintenance of optimum growth and transport media along with tight regulation of temperature.[5,8] Microbiological assays have indicated that prolonged time periods of storage and transportation can lower the efficacy of H. pylori culture by ~6.6% while higher growth temperatures can significantly reduce the positivity rate of H. pylori culture and sensitivity from 37% to 24%.[9] To address the discrepancy associated with the efficacy of H. pylori culture testing, this study was designed for an

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accurate comparison with the gold-standard technique of endoscopic histopathology.

METHODOLOGY

This study was designed as a cross-sectional study and conducted at the Gastroenterology Department of the Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, over a period of 9 months from January to September 2021, after obtaining Ethics Review Committee permission via letter number. The sample size came out to be 70 as calculated by WHO sample size calculator, by taking confidence interval 95%, margin of error 5% and estimated prevalence of H pylori as 55-95% in Pakistan.[10] The study participants were enrolled using non-probability consecutive sampling.

Inclusion Criteria: Patients who were older than 18 years, had a history of chronic dyspepsia, and were abstinent from cigarette smoking and alcohol for at least 5 years, were included in the study.

Exclusion Criteria: Individuals who had used any PPI therapy in the past 2 weeks were excluded.

Patients who had a history of dyspepsia for 12 weeks or longer were categorized as potential cases of chronic dyspepsia. While considering histopathological examination as the gold standard diagnostic parameter, all the patients having a history of chronic dyspepsia were planned to undergo upper GI endoscopy, following which biopsy samples were obtained from the gastric antrum and body. A patient was said to be infected with H. pylori if the following features were isolated upon histopathological analysis of the biopsy specimen: detection of H. pylori, lymphocytic infiltration of the gastric mucosa, glandular atrophy or intestinal metaplasia of gastric epithelial lining. Informed consent was obtained from all the participants after which patients' demographic details as well as their clinical history of dyspepsia were entered into a pre-designed proforma. After preserving half of the gastric tissue specimens in formalin for histopathological analysis, the other 50% of samples were stored in Brucella Broth, and forwarded to the Department of Pathology, PEMH, for microbiological culture. Transportation of biological specimens was carried out at temperature < 24°C and within 24 hours of their withdrawal. All patients were followed in the outpatient department until their gastric biopsy and culture reports were obtained.

The statistical data was analyzed by Statistical Package for the Social Sciences (SPSS) version 23.0. Relative frequencies and percentages were calculated for categorical variables. Mean and standard deviation values were determined for continuous variables. Pearson's chi-square test and independent/unpaired t-test were used to allow a comparison of various parameters between the patients with and without H. pylori. Cohen's kappa coefficient (κ) was also determined to estimate the agreement between H. pylori histopathology and microbiology quantitatively where a p-value ≤ 0.05 was considered as significant.

RESULTS

A total of 73 patients were enrolled into the study, all of whom had a history of chronic dyspepsia. Mean patient age was 45.8±14.72 years, 46(63%) participants were males and 27(37%) were females with average clinical duration of dyspepsia being 7.0±4.0 months. Furthermore, all participants had been consuming PPIs for variable periods of time with as many as 45(62%) participants taking PPI therapy for less than 12 weeks. Table-I enlists the baseline parameters of all enrolled patients with dyspepsia.

Table-I: Baseline Parameters of Patients with Dyspepsia (n=73)

Study Parameters		n(%)
Age (Mean ± SD)		45.8±14.72 years
Gender	Males	46(63%)
	Females	27(37%)
History of Dyspepsia (Mean ± SD)		7.0±4.0 months
Duration of PPI Treatment	< 12 weeks	45(61.6%)
	12-24 weeks	25(34.3%)
	>24 weeks	3(4.1%)
Socioeconomic Status	Lower class	9(12.3%)
	Lower middle class	38(52.1%)
	Upper middle class	17(23.3%)
	High class	9(12.3%)

**PPI: Proton Pump Inhibitor, SD: Standard Deviation*

A comparative analysis of findings obtained from histopathology versus microbiological culture in terms of their relation to patient-related parameters is shown in Table-II. A positive result for H. pylori was seen to be significantly associated with a poor socioeconomic status (p=0.004 for histopathology and p=0.026 for microbiology) and a shorter duration of previously taken PPI therapy (p=0.032 for histopathology and p=0.007 for microbiology).

Cohen's kappa co-efficient was also estimated for the two diagnostic modalities which highlighted only a moderate agreement between histopathology and microbiology (κ = 0.60) with the latter method exhibiting a much inferior sensitivity (66.7%) and specificity (92.6%) with diagnostic accuracy of 78.1% as illustrated by Table-III.

Table- II: Comparison of Diagnostic Techniques with the Baseline Parameters of Patients (n=73)

Study Parameter		Histopathology			Microbiology		
		H. pylori		*p*-value	H. pylori		*p*-value
		Present	Absent		Present	Absent	
Total Patients (n = 73)		46(63.0%)	27(37.0%)	-	34(46.5%)	39(53.4%)	-
Age (Mean years ± SD)		46.3±13.9	44.9±16.2	0.691	43.8±11.8	47.5±16.8	0.280
Gender	Male	29(63.0%)	17(62.9%)	0.995	24(70.5%)	22(56.4%)	0.211
	Female	17(36.9%)	10(37.0%)		10(29.4%)	17(43.5%)	
Socioeconomic Status	Low 9 (12.3%)	6(13.0%)	3(11.1%)	0.004	5(14.7%)	4(10.2%)	0.026
	Lower Middle 38 (52.1%)	29(63.0%)	9(33.3%)		21(61.7%)	17(43.6%)	
	Upper Middle 17 (23.3%)	10(21.7%)	7(25.9%)		8(23.5%)	9(23.1%)	
	High 9 (12.3%)	1(2.1%)	8(29.6%)		0(0.0%)	9(23.1%)	
History of dyspepsia (Mean months ± SD)		6.43±3.7	8.0±4.3	0.116	6.7±4.3	7.3±3.8	0.561
Duration of PPI Therapy	<12 weeks 45 (61.6%)	32(69.5%)	13(48.1%)	0.032	25(73.5%)	20(51.2%)	0.007
	12-24 weeks 25 (34.3%)	11(23.9%)	14(51.8%)		6(17.6%)	19(48.7%)	
	>24 weeks 3 (4.1%)	3(6.5%)	0(0.0%)		03(8.8%)	0(0.0%)	

**PPI: Proton Pump Inhibitor, SD: Standard Deviation*

Table-III: Sensitivity and Specificity of H pylori detection on Microbiological Culture and Histopathology, (n=73)

	Histopathology positive	Histopathology Negative
Culture Positive	32(69.6 %)	2(7.4 %)
Culture Negative	14(30.4 %)	25(92.6 %)

*Sensitivity = TP/(TP+FN)= 32/(32+14)*100 = 66.7%*
*Specificity = TN/(TN+FP)= 25/(25+2)*100 = 92.6%*
*Positive Predictive Value = TP/(TP+FP)*100 = 32/(32+2) = 94.1%*
*Negative Predictive Value = TN/(TN+FN)*100 = 25/(25+1) = 96.1%*
*Diagnostic Accuracy = (TP+TN)/All patients*100 = (32+25)/73 = 78.1%*

DISCUSSION

This study has shown that microbiological culture for H. pylori matches the precision of histopathology only moderately. Despite possessing a fairly high specificity, microbiology reveals a significantly lower value for sensitivity with ~30% of H. pylori positive cases being omitted by culture method. In one cross-sectional study, it was noted that within a sample of 334 individuals, up to 26.3% were labelled as H. pylori positive by using culture and sensitivity technique with both culture and rapid urease testing exhibited a strong correlation having a Cohen's coefficient of 0.81 while culture testing of H. pylori revealed a significantly higher sensitivity of 87% as compared to 69.6% thus making rapid urease testing another reliable method of H. pylori detection.[11] Nonetheless, by considering histopathological examination as the benchmark, the authors determined a relatively comparable sensitivity of 78% for H. pylori culture testing, with their mutual concordance estimated to be 0.76 (11) while in another study, similar results were obtained where microbiological culture and sensitivity accurately isolated H. pylori from a total of 68 (76.4%) patients out of a sample of 89 confirmed cases.[12] In regions with endemic H. pylori, the pathogen largely affects individuals belonging to lower socioeconomic groups while its prevalence is also significantly higher in the developing countries.[13,14] similar to our study where a total of 76% confirmed cases of H. pylori were categorized within low-income groups. Microbiological testing for H. pylori is a lengthy, time-consuming process and it also yields remarkably less reliable results.[15] but microbiological culture and sensitivity holds a potentially useful application in the timely evaluation of bacterial susceptibility to antibiotic treatment.[16] With growing concerns of antimicrobial resistance, it has been recommended that for patients who fail to undergo remission

following treatment with second-line antimicrobials and within regions where antibiotic resistance to clarithromycin-containing triple therapy is expected to be significantly higher (> 15%), culture and sensitivity should be performed to improve the overall prognosis.[10-17] Furthermore, an initial treatment regimen based on microbiological culture results, although not recommended, still stands superior to empirical mode of therapy.[18] The diagnosis of H. pylori has a strong association with poor socioeconomic status as well as total duration of PPI treatment. Given the relative lack of data showing the diagnostic accuracy of microbiology for H. pylori in our native population, the current study highlights and reaffirms the previous global findings pertaining to the inadequacy of microbiology in H. pylori infection.

LIMITATION OF STUDY

The authors recognize that the current research was conducted as a single center study which could potentially undermine its accuracy as compared to multicenter trials. In addition, the authors did not use rapid urease detection in their comparative analysis due to methodological restrictions.

CONCLUSION

In contrast to histopathological testing, microbiological analysis has a potentially lower level of accuracy in the laboratory detection of H. pylori.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

NA & MH: Data acquisition, data analysis, critical review, approval of the final version to be published.

MASK & MFS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

HA & HBT: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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