ORIGINAL ARTICLE

THE DIAGNOSTIC ACCURACY OF H. PYLORI CULTURE AND RAPID UREASE TEST IN THE DIAGNOSIS OF H.PYLORI INFECTION KEEPING HISTOPATHOLOGY AS THE GOLD STANDARD

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ABSTRACT

Objective: The aim of this study was to determine the diagnostic accuracy of biopsy-based tests in the detection of Helicobacter Pylori infections keeping histopathology as a gold standard.

Materials and Methods: This validation study was conducted from August 2018 to February 2019 at Khyber medical college, pathology department, Peshawar, and comprised biopsy samples of 87 patients obtained via endoscopy which was done at the gastrointestinal endoscopy department, Hayatabad medical complex (HMC). Patients with a history of prior antibiotics and PPIs were excluded from the study. Rapid urease test (RUT) and culture of gastroduodenal biopsies were validated against the histopathological examination of gastroduodenal mucosa.

Results: Out of 87 patients, 57.47% (50/87) were male and 42.52% (37/87) were female patients with an age range of 18 -80 years and a mean age of 45 years. Endoscopic findings showed 64.36% (56/87) of patients with gastritis, duodenitis with 6.89% (6/87), peptic and duodenal ulcers with 22.98% (20/87), erosion with 4.59% (4/87) and adenocarcinoma of stomach with 2.29% (2/87). The sensitivity and specificity of culture were 45.45% and 90.7% respectively, while positive and negative predictive values were 62.5% and 83% respectively. The sensitivity and specificity of the rapid urease test (RUT) were 81.81% and 90% while positive and negative predictive values were 90% and 94% respectively.

Conclusion: The sensitivity and specificity of the rapid urease test were 82 and 90% respectively, while that of culture was 45 and 91% respectively.

Keys Words: Helicobacter Pylori, Gastritis, Rapid urease test, Adenocarcinoma

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INTRODUCTION

Helicobacter Pylori is the main cause of most gastrointestinal diseases in humans. Being a fastidious, gram-negative bacterium and possessing microaerophilic nature, it contributes to the pathogenesis of many stomach diseases including chronic gastritis, peptic ulcers, and gastric carcinoma[1]. It also contributes to the development of mucosa-associated lymphoid tissue lymphoma (MALT)[2]. It has been declared by the world health organization as a class-1 carcinogen[3]. Helicobacter Pylori affects fifty percent of the world's population with its associated diseases[2]. The prevalence of infections in developing countries is higher as compared to developed countries and the infection is acquired at an early age[4]. The prevalence of infection in Pakistan is about 92%[3]. The survival of this bacterium in the highly acidic environment of the human stomach is through its utilization of the urease enzyme which hydrolyzes urea to release Carbon Dioxide and Ammonia, thus providing a neutral niche in the gastric mucosa[5]. It also requires motility by means of flagella to penetrate the gastric mucosa. Both factors play an important role in gastric colonization[6]. Fecal oral, oral-oral

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routes, and drinking water are some of the routes of transmission of Helicobacter pylori infections[7,8].

Various diagnostic tests are available for the detection of Helicobacter pylori9. These include invasive tests such as rapid urease test (RUT)10, bacterial culture and histology after obtaining biopsy through endoscopy, and several non-invasive tests which include urea breath test, serology, and stool antigen[11,12]. The choice of diagnostics depends upon the patient's age, complaints, availability of tests in hospitals, cost-effectiveness, and technical difficulty levels. Culturing bacteria, though labor intensive because of the fastidious nature of the bacterium, is a highly sensitive test to detect H.pylori from gastric biopsies. One added advantage of culturing bacteria is the benefit of performing antibiotic susceptibility testing[13]. Histology is another invasive technique that is employed for the direct detection of the organism[14]. Several stains can be employed for the detection of H.pylori like Hematoxylin and eosin (H & E), Giemsa, Warthine-Starry & immune-histochemical stains[15]. Molecular identification of H.pylori by polymerase chain reaction (PCR) has been so far the most reliable method to detect the bacterium from gastric biopsy specimens, saliva, stool, and gastric juice[16,17].

The present cross-sectional study aimed to identify Helicobacter pylori from gastric biopsies obtained through endoscopy, to compare detection with other invasive techniques such as bacterial culture, rapid urease test (pronto dry), and histological detection.

MATERIALS AND METHODS

This validation study was conducted from August 2018 to February 2019 at Khyber medical college, pathology department, Peshawar and comprised of total 87 patients who underwent endoscopy at the gastrointestinal endoscopy department, Hayatabad medical complex (HMC) Peshawar, with symptoms of gastrointestinal disturbances like acid reflux, abdominal pain, heartburn/dyspepsia, and vomiting and the study was approved by the ethical review committee of Hayatabad Medical Complex, Peshawar.

Patients with prior history of intake of antibiotics and proton pump inhibitors (PPIs) were excluded from the study. Informed consent was taken from each patient prior to the endoscopy. A total of 6 biopsy specimens were taken each from the antral and corpus regions of the stomach. For bacterial culture, two specimens were collected in sterile tubes containing 10 ml of thioglycolate broth (TGB), (Oxoid UK), and two of the specimens were collected for histopathology in ependroph tubes with 10% formalin, stored at 4℃. The remaining two specimens were collected for pronto dry rapid urease test. The specimens were transported to Khyber medical college, pathology department within one hour of collection to avoid desiccation and to maintain bacterial motility.

The biopsy specimen was chopped and inoculated on Columbia blood agar supplemented with 10 % sheep blood and DENT supplement (OXOID U.K). The culture plates were incubated at 37℃ for 48-72 hours in an anaerobic jar with Campy-Gen sachets to provide a microaerophilic environment and then observed for colonies to appear. After obtaining colonies from the culture, Gram staining was performed to confirm curved gram-negative rods. Urease test, catalase, and oxidase tests were done for confirmation of Helicobacter pylori. Rapid Urease Test, (RUT) was done using (Pronto Dry, Gastrex, France) which consists of a dry filter paper containing urea, a pH indicator (phenol red), buffer, and a bacteriostatic agent sealed in a plastic slide. A positive test was shown by the change of color of the dot from yellow to pink. The test result was read at 30 minutes and 1 hour after sampling as per the manufacturer's instructions.

Biopsy specimen which was taken for histological identification was fixed in formalin for 24 hours, dehydrated, and then embedded in paraffin wax. Sectioning was done under the microtome and stained with hematoxylin-eosin and Giemsa stains. The slides were read by an experienced histopathologist who was completely blinded to the clinical information. Curved-shaped rods were identified on the mucosal surface and were taken as positive.

Determination of sensitivity and specificity, positive predictive values (PPV), and negative predictive values (NPV) were done for comparative studies. The frequency of Infection was expressed as percentages.

RESULTS

Out of 87 patients, 57.47% (50/87) were male and 42.52% (37/87) were female patients with the age range of 18 -80 years and a mean age of 45 years who underwent endoscopy.

Endoscopic findings revealed 64.36% (56/87) of patients with gastritis, duodenitis with 6.89% (6/87), peptic and duodenal ulcers with 22.98% (20/87), erosion with 4.59%(4/87) and adenocarcinoma of stomach with 2.29% (2/87) as shown in table 1. Total positive H.pylori isolates obtained by bacterial culture were 16(18.39%) while 22(25.28%) of the isolates were detected to be positive for H.pylori by Giemsa and hematoxylin-eosin stain. 18 (20.68%) of the patients were identified as positive for H.pylori on the pronto dry urease test.

In our study, culture, Histopathology and RUT were considered the gold standard for the determination of sensitivity, specificity, and positive and negative predictive values of the tests. At least two of the three tests were taken as positive cases for diagnosis of H.Pylori infections. Out of 87 patients who underwent endoscopy, 4 of the patients were excluded because of negative results in 2 of the 3 invasive tests.

Comparison of culture and Histopathology: The sensitivity and specificity of culture versus histopathology as in our study were 45.45% and 90.7% respectively, while positive and negative predictive values were 62.5% and 83% respectively shown in table 2.

Comparison of Rapid Urease Test and Histopathology: The sensitivity and specificity of RUT versus histopathology in our study were 81.81% and 90% while positive and negative predictive values were 90% and 94% respectively (Table 2).

Table 1: Endoscopic findings of patients (n=87)

Endoscopy	Number of Patients	Percentage
Gastritis	56	64.36%
Duodenitis	6	6.89%
Peptic and Duodenal Ulcers	20	22.98%
Erosions	4	4.59%
Adenocarcinoma of stomach	2	2.29%

Table 2: Comparison of different Invasive tests in the diagnosis of H.P infections

Diagnostic Tests	Sensitivity	Specificity	Positive Predictive Values	Negative Predictive Values
Culture vs. Histopathology	45.45%	90.7%	62.5%	83%
Rapid Urease Test vs. Histopathology	81.81%	96.92%	90%	94%

DISCUSSION

Helicobacter Pylori infections are so common worldwide that half of the world's population is reported to be affected. The prevalence of infection is higher in underdeveloped countries and infection is attained at an early age[18]. For effective management, it is important to do initial diagnosis through invasive gastric biopsy methods including bacterial culture or histopathology with a rapid urease test (RUT) or molecular-based detection of the bacterium. Non-invasive tests like the urea breath test and H.pylori stool antigen test should not be employed in the initial diagnosis of H.pylori infection, although these tests provide good support of evidence in the case when only histopathology is used as an invasive test for diagnosis.

In our study, 22.28% of patients were positive for Helicobacter Pylori. 18.39% of patients were diagnosed by bacterial culture. Culturing of this bacterium is recommended in case of failure of antibiotic treatment. This technique gives an added advantage as antibiotic susceptibility testing can be conducted which helps the clinicians in prescribing appropriate antimicrobial treatment[19ÿ]. 22.28% of patients were diagnosed through histological identification. Rapid urease test was positive in 20.68% of patients. The sensitivity and specificity of culture in comparison to histopathology in our study were 45.45% and 90.7%. This low sensitivity of culture owes to many reasons which include low bacterial count and the presence of morphologically different forms of Helicobacter Pylori which are though viable but otherwise non-culturable. The use of prior antibiotics also contributes to low sensitivity and false negative results. The low sensitivity of culture in our study is in accordance with previous studies[20]. Another study conducted by Cosgun Yasemin et al showed that culture had lower sensitivity as compared to histopathology[11]. The reason for the low sensitivity of culture lies in the difficulty of culturing this bacteria due to its microaerophilic nature, isolation time (5 -7 days), and special growth requirements[21]. In case of clinical indication of performing endoscopy to obtain a gastric biopsy for Helicobacter Pylori-associated gastric diseases, the first choice is the rapid urease test as it provides quick and easy detection of urease activity in the gastric biopsy specimen. This test which was first defined and performed by McNulty and Wise in 1985, has gained recognition due to its high sensitivity in the detection of Helicobacter Pylori in gastric biopsy samples[22]. Many studies have shown the sensitivity of this test between 80 - 99%.[23,24,25]

In our study, the sensitivity and specificity of the rapid urease test as compared to histopathology were 81.81% and 96.92%. The results of our study affirm previous studies in which RUT sensitivity was higher.[26] Study conducted by Dechant, Franz-Xaver, et al also shows that the rapid urease test is more sensitive in comparison to the histopathology of the gastric biopsy for confirmation of Helicobacter Pylori.[27]

An important observation of this study is the significant association of Helicobacter Pylori with gastric adenocarcinoma. A literature review has revealed that there is a substantial risk of progression to gastric adenocarcinoma in patients diagnosed with Helicobacter Pylori gastritis which is why it is suggested that early detection of the bacteria may help in eradication and effective treatment of Helicobacter Pylori which may lead to lower risks of progression to adenocarcinoma of the stomach.[28] In our study, the percentage of adenocarcinoma was 2.29%.

Owing to the small size of the study population, our study may not reflect the actual burden of Helico-

bacter Pylori-related diseases. Another limitation was the difficulty in culturing viable bacteria due to the unavailability of a CO2 incubator.

CONCLUSION

The present study suggests that Helicobacter Pylori remains a leading cause of many gastrointestinal diseases including peptic ulcers, duodenitis, gastric erosions, and adenocarcinoma of the stomach. The sensitivity and specificity of the rapid urease test were 82 and 90% respectively, while that of culture was 45 and 91% respectively.

In order to get an actual frequency of Helicobacter Pylori-related gastric diseases, a study of longer duration with a large study population is required.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Ali M:	Conception, analysis, interpretation, discussion
Nadeem S:	Literature search, Data collection, analysis
Gul A:	Writing, Proof Reading, Critical appraisal
Waseem H:	Statistical analysis and data handling
Ihsanullah:	Statistical analysis and bibliography
Qudrat A:	Literature search, write up

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.