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DETECTION OF *HELICOBACTER PYLORI* FROM THE GASTRIC BIOPSY SAMPLES OF THE SUSPECTED PEPTIC ULCER DISEASE PATIENTS BY CULTURE AND NESTED PCR

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ABSTRACT

Background: One of the most prevalent persistent human illnesses is caused by *Helicobacter pylori* (*H. pylor*i). Peptic ulcer disease, gastric cancer, and gastric mucosa-associated lymphoid tissue lymphoma are all firmly linked to the infection, which plays a crucial role in the progression of various gastro-duodenal disorders. **Aim:** Our purpose was to examine the diagnostic compatibility of culture and Nested PCR and to create a useful comparison. **Method:** Patients undergoing upper gastrointestinal endoscopy at the Gastroenterology Department of the Chittagong Medical College and Hospital, Chattogram were included if they met the inclusion and exclusion criteria. The research was conducted from January 2021 to December 2021 by the Department of Microbiology at Chittagong Medical College in Chattogram. A total number of 110 individuals with dyspeptic symptoms, who underwent upper GI endoscopy, were included. **Results:** Two stomach antrum samples from each patient were taken for culture and Nested PCR analysis independently. Of the 110 patients tested for PUD, 74 (67.27%) were positive for cultures, whereas 32 (32.73%) tested negative. 82 (74.55%) tested positive by Nested Polymerase Chain Reaction, and 28 (25.45%) did not. **Conclusion:** Nested PCR was shown to have a greater sensitivity (97.30%) and accuracy (89.09%) than traditional culture method for detecting *H. pylori* infection, as shown in the research. In addition, PCR is sensitive, fast, and yields results rapidly.

INTRODUCTION

Helicobacter pylori, a prevalent bacterium in over half of the world's population, has gained recognition for its substantial role in gastrointestinal diseases like gastritis, peptic ulcer disease, and gastric adenocarcinoma.[1-2]

Transmission of *H. pylori* occurs through oral-oral and fecal-oral routes, potentially including gastro-oral transmission through reflux or regurgitation of stomach contents.[3] Once acquired, the infection persists for extended periods, with humans as the sole reservoir.

Timely and accurate diagnosis is paramount for dyspeptic patients due to the association between *H. pylor*i and gastro-duodenal diseases. The NIH Consensus Development Panel stresses the need for diagnosis before therapy, emphasizing invasive and non-invasive diagnostic tests, their choice governed by factors like availability, clinical context, cost, and the presence of confounding factors.[4] Delayed or incorrect diagnosis not only heightens morbidity but also leads to economic losses and, in severe cases, mortality, especially in malignant patients.[5]

Diagnostic methods vary from invasive (requiring endoscopy) to non-invasive options such as urea breath tests, serological tests, and stool antigen tests. The choice depends on factors like sensitivity, specificity, cost, and availability. Invasive methods like culture, rapid urease tests, histopathology, and PCR require endoscopic examination of gastric mucosa, whereas non-invasive methods offer different accuracy levels.[6] Nested PCR, known for its sensitivity and specificity in detecting *H. pylori* DNA, surpasses culture method.

Accurate biopsy site selection, favoring the antrum in most cases and the corpus for specific conditions, is crucial to avoid false-negative results. Culture methods, while effective, exhibit variable sensitivity due to the bacterium's fastidious nature, recovering it from 50% to 70% of infected biopsies in experienced labs.

Employing PCR technology, particularly Nested PCR targeting the ureA gene for its high sensitivity and species specificity, allows not just detection but also characterization of *H. pylori* genes and antimicrobial resistance mutations.[7-9]

This research undertook both culture and Nested PCR methods to detect *H. pylori* in gastric biopsy samples from dyspeptic patients. The study aimed to compare and assess the diagnostic efficiency of these techniques in identifying *H. pylori* infection.

METHODOLOGY

The study was conducted at Department of Microbiology, Chittagong Medical College, Chattogram over one year (January to December 2021) adopted a cross-sectional descriptive design. It involved suspected Peptic Ulcer Disease (PUD) patients exhibiting dyspepsia, both outpatient and admitted cases in the Gastroenterology Department at CMCH. The research utilized gastric antral biopsies and initially targeted a sample size of 340, although constraints related to COVID-19 and time limitations led to a reduced sample of 110 through purposive sampling.

Inclusion criteria involved patients showcasing PUD clinical features and endoscopically confirmed gastritis/ulcer/erosion. Exclusions covered those receiving specific medications or therapies within defined timeframes, individuals with active bleeding/perforation/ulcers, or regular NSAID or steroid users with certain co-morbid conditions. Patients also needed to provide biopsy consent.

Data collection involved a structured questionnaire, and subsequent analysis employed SPSS version 20. Laboratory procedures included various tests for *H. pylori* detection, comprising culture, microscopic examination, biochemical assays, and Nested PCR, all conducted on gastric biopsy samples from the antrum.

The PCR process involved DNA extraction from biopsy samples using a QIAGEN DNA Mini Kit, followed by amplification using specific primer sets targeting the ureA gene. Gel electrophoresis facilitated the visualization of amplified DNA products under UV light.

Statistical analysis encompassed descriptive statistics for continuous and categorical variables, comparison through $\chi 2$ tests, and calculation of sensitivity, specificity, positive and negative predictive values, and accuracy of the Nested PCR method in detecting *H. pylori* concerning the culture method. The significance level was set at $P < 0.05$ with a 95% confidence interval.

The laboratory procedures for *H. pylori* detection involved culture methods, growth assessment based on colony characteristics, microscopic examination, and biochemical tests such as urease, oxidase, catalase, and motility tests. Additionally, Nested PCR utilizing specific primer sets targeting the ureA gene was conducted for *H. pylori* detection.

The findings of this study, extracted from biopsies via various laboratory techniques, were analyzed and compared for their efficacy in detecting *H. pylori* infection among dyspeptic patients with suspected PUD.

RESULTS

Table 01: Distribution of age and sex among the study subjects (n = 110).

Age in Groups	Male	Female	Total (%)
20- 40 Years	36 (32.2)	24 (21.8)	60 (54)
40 – 60 Years	18 (16.4)	17 (15.5)	35 (31.9)
> 60 Years	10 (9.1)	5 (4.5)	15 (13.6)
Total	**64 (58.18)**	**46 (41.82)**	**110 (100.0)**

Figures within parentheses indicate percentages

Mean age: 39.56 ± 14.38 years

Table-1 shows age and sex distribution among the study subjects where 54% belong to 20-40 years, whereas 31.9% belong to 40-60 years old. Besides 58.18% were male and 41.82% were female with male to female ration 1.4:1.

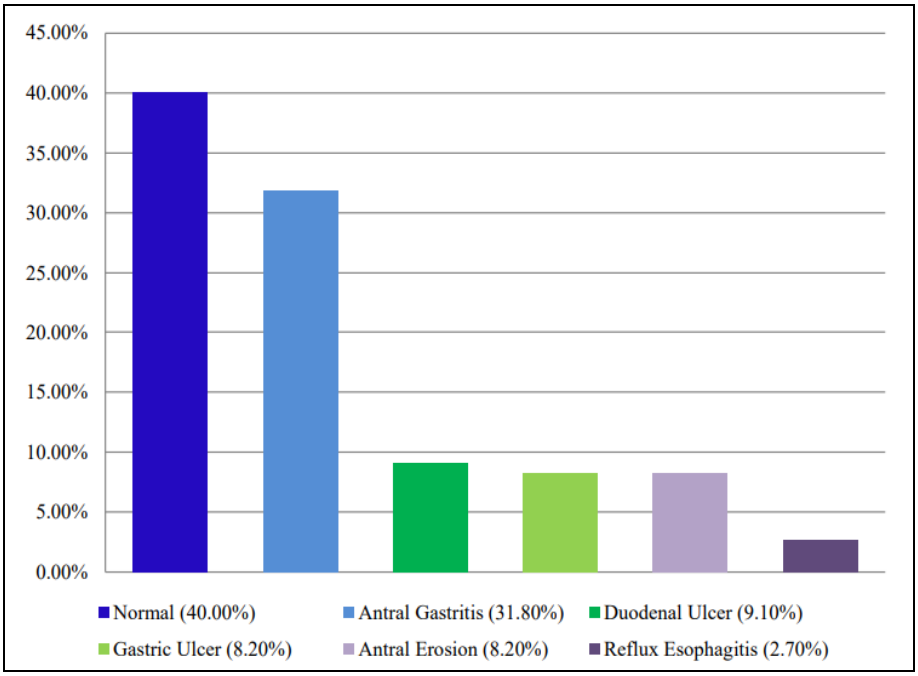

Figure 01: Bar chart showing distribution of endoscopic findings.

In figure-1 Bar chart showed 31.80% had antral gastritis, 9.10% had duodenal ulcer, 8.20% had gastric ulcer and 2.70% had reflux esophagitis.

Table 02: Showed Result of Culture for the detection of *H. pylori* (n=110).

	Result	No. of subject	Percentage (%)
Growth *of H. pylori*	Positive	74	67.27%
	Negative	36	32.73%
	Total	110	100%

Table-02 showed among 110 patients, 67.27% were positive for *H. pylori* culture.

Table 03: Result of Nested PCR for the detection of *H. pylori* (n=110).

	Result	No. of subject	Percentage (%)
Nested PCR	**Positive**	82	74.55%
	Negative	28	25.45%
	Total	110	100%

Table-03 showed among 110 patients, 82 (74.55%) were PCR positive and 28 (25.45%) were PCR negative.

Table 04: Comparison of the result as per different diagnosis methods (Culture and Nested PCR).

Culture +ve PCR +ve	Culture +ve PCR –ve	Culture – ve PCR +ve	Culture –ve PCR –ve
72	02	10	26

Table-04 showed 72 cases were both culture and PCR positive and 26 cases were both culture and PCR negative. 02 were culture positive but PCR negative whereas 10 were culture negative but PCR positive.

Table 05: Association between Culture and Nested PCR test results.

Culture	PCR			
	Positive	**Negative**	**Total**	
Positive	72 (87.80)	02 (7.10)	**74**	χ^2 = 58.074 P = < 0.001 **VHS**
Negative	10 (12.20)	26 (92.90)	**36**	
Total	**82**	**28**	**110**	

- Figures within parentheses indicate percentages
- VHS = Very Highly Significant (P < 0.001)

Table-05 showed association between culture and Nested PCR test results by $\chi 2$ test ($\chi 2$= 58.074) and the result is very highly significant with p value <0.001. In our study, among 74 culture positive cases, 72 were PCR positive whereas among 36 culture negative cases, 10 were PCR positive. Among 74 culture positive cases, only 02 were PCR negative (false negative).

Table 06: Sensitivity, Specificity, PPV, NPV and Accuracy of Nested PCR method.

Statistic	Value	95% CI
Sensitivity	97.30%	91.70% to 99.50%
Specificity	72.20%	60.80% to 76.80%
Positive Predictive Value (PPV)	87.80%	82.80% to 89.80%
Negative Predictive Value (NPV)	92.90%	78.20% to 98.70%
Accuracy	89.09%	81.60% to 92.10%

Table-06 showed high sensitivity (97.30%), specificity (72.20%) and accuracy (89.09%) of Nested PCR method

with Positive predictive value (PPV) 87.80% and Negative predictive value (NPV) 92.90%.

DISCUSSION

H. pylori infection is one of the most frequent chronic infections in human being globally and closely related with peptic ulcer disease and stomach cancer. 8 Infection occurs once the bacteria is ingested by the mouth. The prognosis of peptic ulcer disease has been found to improve after *H. pylor*i eradication. For efficient treatment and management of the condition, a correct diagnosis of infection is necessary to prevent complications and recurrence.[9]

The diagnostic accuracy for *H. pylori* infection is variable depending on test sensitivity, specificity, availability, speed, and cost.[10] Urea breath test, serology, or stool antigen test are all examples of non-invasive diagnostics. Culture, histopathological examination, the rapid urease test, and the polymerase chain reaction (PCR) technique are all invasive methods for detecting *H. pylori.* An *H. pylori*-specific serological test may identify infection, but it cannot differentiate between a current infection and a past exposure to *H. pylori* infection. There is a potential of a false positive result with both the urea breath test and the stool antigen test, despite their excellent sensitivity and specificity. Culture demonstrates specificity but lacks sensitivity which is comparable to histopathological investigation. More false negatives occur in the rapid urease test. Although nested PCR outperforms other approaches in terms of sensitivity and specificity, it is labor-intensive and needs trained personnel.

The majority of the 110 patients in this research who were experiencing upper GIT symptoms were young adults (mean age 39.56 ± 14.38 years). The male-to-female ratio in this research was close to what was reported by Ogihara *et al*; 2000.[11] According to the age distribution of the patients in our research, the vast majority of participants were in their thirties and forties. Patients younger than 40 years old had a greater prevalence of *H. pylori* (54%) compared to those between the ages of 40 and 60 years old (31.9%). In more than 60 years, the rate of occurrence decreased. While previous research by Ahab *et al;* 2015 indicated that *H. pylori* prevalence increased with age, our study found the opposite to be true. This discrepancy may be attributed to a decrease in the specific serological response among older patients due to a reduction in the number of microbes as a result of gastric atrophy.[12]

Endoscopic results in our investigation varied from normal (40%), to antral gastritis (31.80%), to duodenal ulcer (9.10%), to stomach ulcer (8.10%), to antral erosion (2.70%), to reflux esophagitis (8.10%). From a group of 72 patients with dyspepsia, Ghosh *et al;* 2014 found that 55.60% had normal endoscopic results, 22.20% had antral gastritis and stomach erosion, 8.30% had reflux oesophagitis, 5.70% had duodenal ulcers, and 2.80% had gastric ulcers.

Our 67.27% culture positive rate for *H. pylori* infection is comparable to the 69% rate reported by Patel *et al;*, 2014. Isolation rates of *H. pylori* using the culture approach were reported 55% in another research conducted by Islam *et al;* 2013.[13]

Negative results for *Helicobacter pylori* infection can be caused by a number of different things, including the presence of the non-culturable coccoid form of the bacterium, the absence or low density of bacterium in the biopsy specimens, the use of antimicrobials and PPIs, improper transport conditions, or the loss of viability of the microorganism due to oxygen exposure.[14]

Our results for the sensitivity and specificity of Nested PCR (97.30% and 72.20%) are quite close to that of Ramis *et al;* 2012 (100% and 75%). 10 Highly sensitive detection of *H. pylori* DNA in a variety of clinical samples is possible using polymerase chain reaction (PCR), which also yields valuable information on the presence of genes encoding particular virulence factors and drug resistance. If the potential for contamination is minimized and diagnostic tools are at hand, PCR may be utilized as the gold standard. Using a Nested or Semi-Nested technique in PCR may boost its sensitivity. Our research showed that just 02 out of the 74 culture-positive cases were PCR-negative, whereas 10 out of the 28 culture-negative cases were PCR-positive. Crossover contamination, the detection of DNA from dead bacteria, and insufficient cleaning of the endoscopes are all potential sources of false positive findings.[14]

H. pylori may be detected using a number of genes with varying degrees of sensitivity and specificity, including 16S rRNA, 23S rRNA, babA, vacA, cagA, ureA, ureB, and ureC genes. Urease enzyme of *H. pylori* seems to be essential for the microbe's survival in the harsh, acidic conditions of the stomach. There are numerous different forms of the urease enzyme. In our research, we have selected ureA gene since it is a species-specific. The Nested PCR technique was used to identify *H. pylori* in stomach samples in a research by other study.[15]

Our research showed that the sensitivity, specificity, PPV, NPV, and accuracy of Nested PCR were, in order : 97.30%; 72.20% & 87.80%; 91.90%; 89.09% respectively. Results from the study by Ramis *et al;* 2012 showed sensitivity, specificity, PPV, NPV of Nested PCR were 100%, 75%, 66.70%, and 100% respectively.[10]

Other study revealed substantial variability of *H. pylor*i genes and suppressed genes in numerous strains, all of which might compromise the specificity of PCR.[15]

Therefore, detecting *H. pylor*i infection with Nested PCR is preferable to the traditional culture approach. Among 110 participants in our research, we found that Nested

PCR was more effective than the culture method (67.27%).

CONCLUSION

Culture is the gold standard for determining *H. pylori* infection, but it is time-consuming, costly, and challenging to execute in a facility with limited resources. Nested PCR was more sensitive (97.30%) and accurate (89.09%) than the standard culture method. Thus, Nested PCR is a valid method for the early diagnosis of *H. pylori* infection.

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