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Molecular Identification of Helicobacter Pylori from Gastric Biopsy Specimens at A Tertiary Care Centre

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Abstract— Background: Helicobacter pylori infection is highly prevalent in developing countries and is a major cause of peptic ulcer, gastric adenocarcinoma, and mucosal-associated lymphoid tissue (MALT) lymphoma. Despite the availability of various assays for confirming H. pylori infection, achieving accurate diagnosis remains challenging due to the limitations of the assays used. This study aimed to evaluate the diagnostic accuracy of Nested PCR with a new, highly specific, and sensitive primer pair, in comparison to the commonly performed Rapid Urease Test (RUT). Methods: Gastric biopsy samples were collected from patients with findings suggestive of H. pylori infection during endoscopy. A Rapid Urease Test was performed on these samples. Subsequently, DNA extraction was conducted, followed by amplification using two primer pairs (Pylo A and Pylo AN) designed from the 16S rRNA gene of the H. pylori genome. Results: Forty eight patients (80%) out of 60 were found to be positive for Helicobacter pylori. Among 25 patients with RUT-negative results, thirteen were found positive by Nested PCR. Conclusions: The results demonstrate that Nested PCR offers higher sensitivity and specificity compared to the Rapid Urease Test for detecting H. pylori in gastric biopsy samples. Therefore, due to its superior diagnostic accuracy, Nested PCR should be regularly used for patients with gastroduodenopathy to prevent potential misdiagnosis.

Index Terms— Helicobacter pylori, Rapid urease test, Nested PCR

I. INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that plays a crucial role in causing gastrointestinal conditions such as peptic ulcers, low-grade B-cell lymphoma (MALT lymphoma), and gastric cancer[1,2]. Numerous epidemiological studies have shown that people infected with H. pylori have a higher risk of developing gastric carcinoma[3]. The prevalence of H. pylori varies greatly across different populations and countries, influenced by socioeconomic factors. In developing countries, the prevalence is around 90%, whereas in developed nations, it is about 50%[4,5]. Furthermore, gastric cancer and peptic ulcers result in over a million deaths globally each year, highlighting their importance as major public health concerns[6,7].

Diagnostic methods for H. pylori include both invasive and non-invasive techniques, utilising either direct or indirect approaches. Direct methods involve microscopic detection and bacterial culture, while indirect methods include urease production tests, stool antigen detection, and antibody detection, which serve as indicators of the body's response to the infection. Advancements in molecular techniques are now commonly used as they offer enhanced sensitivity and specificity in diagnosing infectious diseases[8]. However, due to resource constraints, methods such as the urea breath test or invasive bacterial culture from biopsied tissue are not feasible in our setting. Additionally, the reliability of immunological tests is frequently questioned.

In recent years, the use of molecular methods like polymerase chain reaction (PCR) has revolutionised diagnostic approaches for detecting H. pylori. Additionally, PCR allows for tracking various genetic changes in the bacteria, aiding in the understanding of drug resistance characteristics[9] and coinfection with other pathogens in gastric diseases[10].

The molecular approach has enabled comparative analyses between traditional methods such as microscopy and the rapid urease test (RUT) with PCR in resource-limited settings, thereby improving the effectiveness of diagnosis and treatment. In our setting, by utilising available molecular methods, we compared RUT and PCR to evaluate the efficacy of each method, contributing to a more comprehensive assessment of the study. Identifying H. pylori infection in gastroduodenal diseases is crucial to preventing potential gastrointestinal malignancies. In developing countries like India, the prevalence of H. pylori is significantly higher in cases of duodenal ulcer, gastric ulcer, and gastritis. Therefore, this study aimed to evaluate the efficacy of two techniques,

Nested PCR and RUT, in detecting H. pylori in gastric biopsy specimens.

II. METHODS

Gastric biopsy samples were obtained from 60 patients (38 males and 22 females) aged 20-69 years who underwent endoscopy for upper gastrointestinal complaints at the Gastroenterology Department of PBM Hospital, affiliated with Sardar Patel Medical College. Patients who had taken non-steroidal anti-inflammatory drugs, bismuth compounds, proton pump inhibitors, oral anticoagulants, or antibiotics effective against H. pylori within the previous two weeks were excluded. Additionally, individuals who had recently undergone blood transfusions, gastric surgery, or had bleeding diathesis were also excluded from the study. From patients showing findings commonly associated with H. pylori infection (antral gastritis, gastric and duodenal ulcers), two gastric biopsy specimens were collected from the antrum of the stomach. One sample was used for the Rapid Urease Test (RUT) performed chairside in the endoscopy room, while the other sample was immediately frozen at -40°C in the Gastroenterology Department for later use in PCR after being transferred to the Clinical Work Laboratory of Sardar Patel Medical College.

One specimen was rapidly examined for the presence of H. pylori using the RUT Card (Gastro Cure Systems, Kolkata, India). In the RUT, the biopsy material was placed into a gel containing a pH indicator that changed color from yellow to red/pink within 2-10 minutes if H. pylori was present, due to the production of ammonia by the organism's urease enzyme.

DNA extraction was performed on the other specimen following the Transiome Genomics (Ahmedabad, India) DNA Purification Kit protocol. DNA extracts were stored at -20°C until used for PCR. An oligonucleotide sequence, Pylo A & Pylo AN from the 16s rRNA of the H. pylori genome, was chosen and synthesised by Bioserve Biotechnologies Company (Hyderabad, India) [11].

Five microliters of each DNA extract sample underwent a two-step nested PCR using two primer pairs from the 16s rRNA gene of the H. pylori genome. The outer primer pair, Pylo A, consisted of 5’-TTGATCCTGGCTCAGAGTGAACG-3’ and 5’-TGCAGCCTACAATCCGAACTGAG-3’, and it amplified a 1274-bp product. After an initial denaturation at 96°C for 5 minutes, the amplification cycle included 40 cycles of 95°C for 1 minute, 56°C for 1 minute, and 74°C for 1 minute. The final cycle included an extension step at 74°C for 5 minutes to ensure full extension of the product.

Following the first round of PCR, 1 microliter of the reaction mixture was transferred to the second round reaction mixture, which contained 0.6 µM of each inner primer and the same buffer as in the first round. The nested inner primer pair, Pylo AN, consisted of 5’-GGTGGAATTCTTGGTGTAGGGGT-3’ and 5’-TAGCATCCATCGTTTAGGGCGTG-3’, and it amplified a 160-bp product. The amplification cycle for the second round of PCR was the same as the first. Ten microliters of the final PCR product were electrophoresed on a 1.2% agarose gel containing 0.5 µg of ethidium bromide per ml.

III. RESULTS

Table 1 & 2 presents the results obtained with RUT and Nested PCR used for the detection of H. pylori infection in gastric biopsy samples. Of the 60 biopsy samples, 35 were positive (58.3%) and 25 were negative (41.6%) by Rapid Urease Test. Forty eight patients (80%) were found to be positive for H. pylori by nested PCR and twelve (20%) were found negative. Out of the 25 patients with RUT-negative results, thirteen were found positive by nested PCR.

In this study, the combination of diagnostic methods RUT & N-PCR increased the test positivity from 58.3% (35/60) to 80% (48/60). In McNemar’s analysis, it was found that the H.pylori RUT results were 78.3% ({35+12}/60) in agreement with Nested PCR results whereas 21.7% results between both diagnostic tests were in disagreement.

The Nested PCR is superior in diagnosing the presence of bacteria in gastric biopsy tissues than the Rapid Urease Card test. However, the agreement between both the assays shows them as of comparable diagnostic efficiency.

Table 1. Results of Rapid Urease Test to detect H. pylori

Total (n)	**60**
RUT Positive	35 (58.3%)
RUT Negative	25 (41.6%)

Table 2. Results of Nested PCR to detect H. pylori

Total (n)	**60**
Nested PCR Positive	48 (80%)
Nested PCR Negative	12 (20%)

IV. DISCUSSION

To diagnose H. pylori infection, a variety of methods are available, and the optimal choice should be based on factors such as sensitivity, specificity, clinical condition, availability, and cost. Consequently, numerous studies have compared and correlated different H. pylori detection methods, both invasive and non-invasive.

In the current study, we compared the molecular method targeting the 16S rRNA gene with the Rapid Urease Test (RUT). The sensitivity of these methods can be influenced by several factors: the number of biopsies taken, the bacterial density in each biopsy, the presence of H. pylori in endoscopic material, and the presence of other microorganisms besides H. pylori[12].

In this study, the sensitivities of the Rapid Urease Test (RUT) and nested PCR were evaluated. Both methods require biopsy samples, but not all biopsies contain a sufficient quantity of H. pylori organisms for accurate detection. While one-step PCR has limited sensitivity, nested PCR has demonstrated superior specificity and sensitivity in detecting H. pylori[13]; thus, it was employed in this study. Nested PCR can be applied to various biological samples, including gastric tissue, saliva, and feces, as long as DNA extraction from the sample is feasible. In our study, nested PCR exhibited high sensitivity (80%), whereas RUT showed lower sensitivity (58.3%). False-negative results with RUT may occur when only a small amount of H. pylori is present, which might be detected by nested PCR.

Lin et al. reported that among 82 gastric biopsy samples, 56 tested positive by RUT and 52 by PCR, suggesting that PCR could complement RUT[14]. A comparison of PCR, histology, culture, and RUT methods revealed that PCR had the highest diagnostic sensitivity (99.4%) for detecting H. pylori infection[15]. Archimandritis et al. also found that the RUT was less sensitive than histology for diagnosing H. pylori infection[16]. Although histological examination was not performed in this study, it is noted that, compared to RUT, PCR is more sensitive for detecting H. pylori infection after treatment[17].

V. CONCLUSION

The Rapid Urease Test (RUT), being a quick and cost-effective method, can be routinely used as a screening tool for detecting H. pylori in many patients during endoscopy procedures. However, the success rate of detecting H. pylori using Nested PCR, at 80%, was higher compared to RUT. While molecular methods such as PCR and Nested PCR have not traditionally been part of routine H. pylori diagnosis, their use has been increasing due to their high sensitivity and specificity. This study concludes that the PCR assay with nested primers is a highly specific and sensitive method for detecting H. pylori DNA in gastric biopsy samples. In the absence of a definitive gold standard for identifying H. pylori, using both RUT and Nested PCR together could help reduce the incidence of false-negative results for H. pylori infection.

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Conflict of Interest

None

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