

STUDY OF DIAGNOSTIC TESTS TO DETECT *HELICOBACTER PYLORI* IN ACID PEPTIC DISEASE

## Microbiology

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## ABSTRACT

**Introduction:** *Helicobacter pylori* is considered to play a major role in the pathogenesis of several gastrointestinal diseases including gastric ulcer, duodenal ulcer, gastric Mucosa Associated Lymphoid Tissue (MALT) lymphoma and distal gastric cancer. This study was conducted to compare different diagnostic methods like Gram's stain, Rapid Urease Test (RUT) and Serology in the diagnosis of *H.pylori* infection. **Methodology:** 100 patients with dyspeptic symptoms who are referred to endoscopic examination were enrolled in the study. Two tissue biopsy samples were taken from the antrum for Gram's stain and RUT. 5ml of blood from each patient was collected and screened for IgG antibodies. **Results:** Out of 100 patients, RUT was positive in 34 cases, Gram's stain was positive in 17 cases and 44 cases showed IgG serology positive. Maximum positivity was found by RUT + Serology (30 cases) followed by RUT + Gram's stain (16 cases) and Gram's stain + RUT + Serology (16 cases). **Conclusion:** Rapid Urease Test + Serology showed highest sensitivity and specificity. So, it was the best combination of tests to confirm an active *H.pylori* infection in a patient.

## KEYWORDS

*Helicobacter pylori* (*H.pylori*), Rapid Urease Test (RUT), Gram's stain, IgG serology.

## INTRODUCTION

*Helicobacter pylori* was discovered in the year 1982 by two Nobel laureates from Australia, John Robin Warren and Barry James Marshall. They proved that there is an association which exists between *H.pylori* and Peptic Ulcer Disease (PUD). *H.pylori* is now recognized as one of the most common bacterial infections in humans.<sup>1</sup> *H.pylori* is considered to play a major role in the pathogenesis of several gastrointestinal diseases including gastric ulcer, duodenal ulcer, gastric mucosa associated lymphoid tissue (MALT) lymphoma and distal gastric cancer.<sup>2</sup> In 1982 Marshall and Warren isolated a spiral or curved urease producing organism in the interface between gastric epithelial cell surface and the overlying mucus gel and it was named as *H.pylori*.<sup>3</sup> Experiments demonstrated that these bacteria can colonize the human stomach thereby inducing inflammation of the gastric mucosa.<sup>4</sup>

There are several tests available for the diagnosis of *H.pylori* infection consisting of invasive tests i.e., rapid urease test, histopathology, bacterial culture, PCR and non-invasive tests i.e., serology, <sup>13</sup>C Urea breath test, stool antigen and *H.pylori* antibody in urine.<sup>5,6</sup>

This study was conducted in the Department of Microbiology, SVMC, Tirupati, with the approval from the Institutional Ethics Committee to compare the different diagnostic methods like Gram's staining, Rapid urease test and serology in the local population to select the most sensitive, reliable and cost effective method for the diagnosis of *H.pylori*.

## MATERIALS AND METHODS

Patients with dyspeptic symptoms who are referred for endoscopic examination were enrolled in the study. The present study has been carried out on 100 patients of different ages and sex from March 2019 to March 2020.

Two tissue biopsy samples were taken from each patient from antrum (within 2cm from pylorus) for microbiological examination. In endoscopy room one biopsy sample is immediately placed in rapid urease test broth and incubated at 37°C for rapid urease test.<sup>7</sup> The tube was examined after 15, 30, 60 minutes and upto 2 hours. A colour change from yellow to red or magenta pink was read as positive. With second biopsy sample material, direct impression smears were

prepared on clean glass slide and were stained with Gram's stain. Gram's stain shows Gram negative, spiral or 'S' shaped organisms with bluntly round ends, size of 0.5-1.0µm wide and 2.5-5.0µm long.

During endoscopic procedure according to the gastroenterologist, endoscopic findings were noted for each case whether it is simple gastritis (erosion) over antrum, gastric ulcer or duodenal ulcer.

5-6ml of blood collected from the same patients and serum was separated and used for the detection of IgG antibody by Anti *H.pylori* IgG ELISA kit. A case was considered positive for *H.pylori* infection if 2 out of the above 3 tests were positive.

## RESULTS

A total of 100 patients participated in the study out of which 66 are males and 34 are females. Majority of cases are in the age group of 40-60 years i.e., 50%. Out of 100 cases Gram's stain showed the bacilli in 17 cases, Rapid Urease test was positive in 34 cases and 44 were positive for anti *H.pylori* IgG antibodies (Table 1).

Table 1: Results of different laboratory tests employed in 100 cases

| Diagnostic test   | No. of positive for <i>H.pylori</i> | No. of negative for <i>H.pylori</i> |
|-------------------|-------------------------------------|-------------------------------------|
| Rapid Urease Test | 34                                  | 66                                  |
| Gram's stain      | 17                                  | 83                                  |
| IgG serology      | 44                                  | 56                                  |

Among different combination of tests, maximum positivity is found by Rapid urease test and Serology i.e., 30 cases followed by Rapid Urease test and Gram's stain i.e., 16 cases and Gram's stain, Rapid Urease test and Serology i.e., 16 cases (Table 2).

Table 2: Positivity for *H.pylori* with different combination of tests

| Test                          | No. of positive patients |
|-------------------------------|--------------------------|
| Rapid Urease test (RUT)       | 34                       |
| RUT + Gram's stain            | 16                       |
| RUT + Serology                | 30                       |
| Gram's stain + RUT + Serology | 16                       |

*H.pylori* infection detection in patients with different endoscopic findings considering Rapid Urease test and Serology as a marker of

infection shown maximum percentage of positivity in duodenal ulcer cases (50%) followed by gastric ulcer cases (35%) and gastritis cases (18.5%) (Table 3)

**Table 3: *H.pylori* infection detection in patients with different endoscopic findings considering RUT+ Serology as a marker of infection**

| S.No. | Endoscopic findings              | Total      | H.pylori positive (RUT+Serology) | H.pylori negative (RUT+Serology) |
|-------|----------------------------------|------------|----------------------------------|----------------------------------|
| 1     | Gastritis (erosions over antrum) | 54         | 10 (18.5%)                       | 43                               |
| 2     | Gastric ulcer                    | 20         | 7(35%)                           | 13                               |
| 3     | Duodenal ulcer                   | 26         | 13(50%)                          | 14                               |
|       | <b>Total</b>                     | <b>100</b> | <b>30</b>                        | <b>70</b>                        |

Among various diagnostic tests Rapid Urease test, IgG serology show 100% sensitivity and Gram's stain shows highest specificity (98.5%) (Table 4).

**Table 4: Statistical analysis of various diagnostic tests for *H.pylori* tested against RUT+Serology**

| Test         | Sensitivity | Specificity | PPV   | NPV   | Accuracy |
|--------------|-------------|-------------|-------|-------|----------|
| RUT          | 100         | 94.5%       | 88.2% | 100%  | 96.1%    |
| Gram's stain | 68.1%       | 98.5%       | 96.7% | 83.3% | 86.9%    |
| IgG serology | 100%        | 83.3%       | 68.1% | 100%  | 87.7%    |

## DISCUSSION

The present study of 100 samples, 34 were Rapid Urease positive, 17 were Gram's stain positive and 44 were positive for anti *H.pylori* IgG antibodies.

Rapid Urease test is simple, rapid and cheap. It is beneficial in tracing the *H.pylori* infection and thus helps us in treating the patients in present time.<sup>8</sup> The present study with 34cases positive for rapid urease test correlates with Nassir E Mohsun et al (35.6%)<sup>9</sup> and nearer to Subbukesavaraja V et al (27%).<sup>8</sup> Rapid Urease test is recommended as the first choice test as result is obtained within 2hours. Sensitivity and specificity of this test was 100% and 94.5% respectively. Sensitivity of this test correlates with study of Ruba S, Abu Sbeih et al (100%)<sup>10</sup> and Aftab et al *H.pylori*epidemiology 2018 (90%)<sup>11</sup> and specificity was more than Safari Mohammed et al (80%). Moreover Rapid urease test is extremely valuable because it gives a positive result for *H.pylori* infection before the patient leaves the endoscopic suit.<sup>12</sup>

Gram's staining is one of the rapid screening procedures for the diagnosis of *H.pylori*. In the present study, 17% cases were positive by Gram's staining and this correlates with Subbukesavaraja Vet al (22.22%) and Rajeswari Pilli et al (18%).<sup>13</sup>

In the present study 44% cases were positive by serological test using ELISA to detect IgG antibodies and this is similar to Rajeswari Pilli et al (42%) and Hoda A Refaay et al (50%).<sup>14</sup> In the present study sensitivity of IgG serology is 100% correlating with Nassir E Mohsun et al (100%) and Ruba S Abu Sbeih et al (100%). Specificity of IgG serology in present study is 83.6%, which is higher than Madhu Sharma et al (63.3%). IgG serology serves as an easy, sensitive, cheap and non-invasive method for screening patients before endoscopy. It is useful in management by virtue of early detection of patients with an increased risk of chronic peptic ulceration.<sup>15</sup>

IgG identification by serological test may indicate active or past infection. Combining Rapid urease test positivity and *H.pylori*antibodies presence in serological test can be taken as a marker for active *H.pylori* infection.

## CONCLUSION

Among all the tests, Rapid urease test + IgG serology showed highest sensitivity and specificity. So it was the best combination of tests to confirm an active *H.pylori* infection in a patient.

If conditions are not favourable for Rapid Urease test + IgG serology, in which Rapid urease test needs invasive procedure of endoscopy and biopsy, then IgG serology alone showed 100% sensitivity and 100% negative predictive value. So it can be used as a primary screening test.

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