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Evaluation of RUT, and ELISA Tests for Detection of *Helicobacter pylori* in Dyspeptic Patients Visiting a Tertiary Care Hospital, South India

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Abstract

Helicobacter pylori **is main causative agent of acute gastritis and peptic ulcer disease. In certain population, this infection leads to gastric cancers viz., adenocarcinoma, and mucosal-associated lymphoid tissue (MALT) lymphoma. The objective of this study was to comparatively evaluate invasive (RUT) and non-invasive (IgM and IgG ELISA) methods for detection of *H. pylori* infection among patients visiting a tertiary care hospital. A total of 285 dyspeptic patients undergoing endoscopic examination were included in this study. From each patient one biopsy specimen and serum samples were collected. Biopsy specimen was subjected to RUT and IgM & IgG ELISA tests were performed using serum samples. *H. pylori* was detected in 127 (44.6%) and 126 (44.2%) cases by RUT and IgM ELISA, respectively. *H. pylori* were detected in 85 (29.8%) samples by IgG ELISA. Based on the combination of RUT and IgM ELISA test, total 128 (44.9%) patients were positive for *H. pylori* infection. Most of the positive cases belonged to 21-40 years age group (60 of 128) followed by 41-60 years age group (31 0f 128). All the three diagnostic methods viz., RUT, IgM ELISA and IgG ELISA used in this study showed a greater prevalence of *H. pylori* infection in female gender compared to male gender. In this study, sensitivity of both RUT and IgM ELISA was similar across different age groups and gender. The advantage of IgM ELISA over RUT is that it does not require endoscopy. Therefore, IgM ELISA could be considered as safe and an alternative method for detection of this pathogen.**

Keywords: *H. pylori*, Detection, RUT, ELISA

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INTRODUCTION

Helicobacter pylori is a gram-negative, fastidious, slow growing bacterium which requires microaerophilic condition for cultivation.[1] It was first discovered by Barry Marshall and Robin Warren.[2] They named this bacterium as *Campylobacter pyloridis*, later renamed as *H. pylori*.[3,4] This bacterium is exclusively human pathogen and infects the gastric mucosa.[5] *H. pylori* is main causative agent of acute gastritis and peptic ulcer disease.[6,7] In certain population, this infection leads to gastric cancers viz., adenocarcinoma, and mucosal-associated lymphoid tissue (MALT) lymphoma.[7] Infection with this bacterium is very common in developing countries (70%-90%) compared to developed countries (25%-50%).[8,9] Prevalence is influenced by many factors such as region, race, age and socioeconomic status. *H. pylori* infection occurs in childhood and if they are not treated bacterial colonization will continue rest of their life.[10] Kids might acquire the infection from the mother by contacting with the contaminated stomach juice.[11] *H. pylori* can be transmitted through feco-oral, gastro-oral and oral-oral routes.[12,13]

H. pylori infection can be treated effectively through antimicrobial therapy. A meta-analysis study provided the evidence that eradication of this bacterium can lead to decrease in the incidence of gastric cancer.[14] Hence, accurate detection of *H. pylori* is crucial to start antimicrobial therapy. Currently available detection methods for *H. pylori* are divided into two categories; invasive, which require endoscopic biopsy and non-invasive. The invasive methods include histopathology and/or immunohistochemistry (IHC), culture, rapid urease test (RUT) and molecular methods. Serology, urea breath testing (UBT), and stool antigen test (SAT) are commonly used non-invasive methods. Each of these methods has merits and demerits. Even after 40 years after discovery, gold standard method for detection of this pathogen is controversial.

Among the techniques for detection of *H. pylori*, the most commonly used technique is RUT. However, to achieve good sensitivity, *H. pylori* load should be at least 10^5 CFU/ml bacteria. Among non-invasive techniques, serology is easy to perform and there are several Enzyme-Linked Immunosorbent Assay (ELISA) kits commercially available for detection of antibodies against this bacterium. The sensitivity of these tests varies according to geographic regions and populations.[15,16] On their own, there is no single test which detects this bacterium with 100% sensitivity and specificity. Hence, these tests are recommended in combinations to achieve better sensitivity and specificity.[17,18] The objective of this study was to comparatively evaluate invasive (RUT) and non-invasive (IgM and IgG ELISA) tests for detection of *H. pylori* among patients visiting a tertiary care hospital.

MATERIALS AND METHODS

Patients and samples

A total of 285 patients with gastric disorders undergoing endoscopic examination at Dr. VRK Women's Teaching hospital & Research Centre were included in this study. This study was conducted between October 2021 to July 2022. The study population consisted of 140 males and 145 females with an age ranging from 9-92 years (mean age = 38 years). Patients who had received antimicrobial therapy, proton-pump inhibitors, non-steroidal anti-inflammatory drugs and H2-receptor blockers, 30 days prior to endoscopy, patients with previous gastrectomy and pregnant or lactating women were excluded from the study. Informed consent was recorded from all patients. One biopsy specimen was obtained from each patient during endoscopy and sera samples were collected for performing IgM and IgG ELISAs.

Rapid Urease Test

RUT was performed using RUT dry test (Gastro Cure Systems, Kolkata, India). Test was performed as per instructions mentioned in kit insert. Briefly, biopsy specimen was introduced into the yellow media of RUT dry test and one drop sterile water was added. Then sticker was covered as before and observed for the dot color change. Result was declared as positive when the color changed from yellow to pink or red within ten minutes.

Enzyme-Linked Immunosorbent Assay

Anti IgM antibodies were detected by ELISA using the *H. pylori* IgM kit (Calbiotech Inc,

California, USA) as per the protocol mentioned in kit insert. Subsequently, Anti IgG antibodies were detected by *H. pylori* IgG kit (Calbiotech Inc, California, USA) as per the protocol given in kit insert. Briefly, 10µl of the serum sample was added to 200µl of sample diluent to prepare 1:21 dilution of test sample. Then, 100µl of diluted patient sera were added into the appropriate wells and incubated for 20 min at room temperature. Wells were washed thrice with 1X wash buffer and blotted on paper towels. Then, 100µl of enzyme conjugate was added and incubated at room temperature for 20 minutes. Washing step was repeated and 100µl of TMB substrate was added and incubated at room temperature for 10 min. Finally, 100µl of stop solution was added and the optical density (OD) was measured within 15 min at 450 nm using ELISA reader.

RESULTS

In this study, total of 285 biopsy samples were collected from patients with gastric disorders undergoing endoscopic examination. According to age, the patients were divided into five groups. Majority of the subjects belonged to 21-40 years age group (131) followed by 41-60 years age group (76) (Table 1). *H. pylori* was detected in 127 (44.6%) and 126 (44.2%) cases by RUT and IgM ELISA, respectively (Table 2). Total 124 (43.5%) samples were both RUT and IgM ELISA positive. *H. pylori* was detected in 85 (29.8%) of 285 samples by IgG ELISA (Table 2).

Based on the combination of RUT and IgM ELISA test, total 128 (44.9%) patients were positive for *H. pylori* infection (Table 2). The age wise and gender wise distribution of RUT, IgM ELISA and IgG ELISA test results were shown in Table 3. Most of the positive cases belonged to 21-40 years age group (60 of 128) followed by 41-60 years age group (31 0f 128) (Table 3). All the three diagnostic methods viz., RUT, IgM ELISA and IgG ELISA used in this study showed that female gender is prone to *H. pylori* infection compared to male gender (Table 2). Sensitivity of both RUT and IgM ELISA was similar across different age groups and gender (Table 3).

Table 1. Distribution of patients in different age groups

Age group	Gender		Total
(Years)	Males	Females	(n)
≤20	27	24	51
21-40	66	65	131
41-60	32	44	76
61-80	13	11	24
81-100	02	01	03
Total	140	145	285

DISCUSSION

Although many tests are available for detection of this pathogen, the gold standard method is still controversial. None of the methods is foolproof and each method has its own advantages and disadvantages with regards to

Table 2. Detection of *H. pylori* by RUT, IgM ELISA and IgG ELISA

Gender	RUT	IgM ELISA	IgG ELISA
Males	56	55	33
Females	71	71	42
Total	127	126	75

Table 3. Distribution of H. pylori cases in different age groups

Age group	RUT		IgM ELISA		IgG ELISA	
(Years)	Males	Females	Males	Females	Males	Females
≤20	12	13	11	12	08	05
21-40	29	31	29	31	18	21
41-60	10	21	10	21	04	14
61-80	03	05	03	06	02	01
81-100	02	01	02	01	01	01
Total	56	71	55	71	33	42

sensitivity, specificity, indication and cost.[19] Hence, it is recommended to use a combination of these tests based on different principles for detection of this bacterium.[17,18] In this study, we have evaluated three diagnostic methods viz., RUT, IgM ELISA and IgG ELISA for detection of *H. pylori*. If *H. pylori* were detected by any of the diagnostic method used in this study it was counted as positive. The sensitivity of RUT was 99.2%, which is in line with several other studies.[20,21] Previous studies indicated that several factors influence the RUT results including the condition of biopsy as well as the disease type. RUT accuracy will depend on site, number, size and *H. pylori* number of biopsy specimen.[22] False negative results may occur, because of antimicrobial therapy or the use of proton-pump inhibitors or irregular distribution of this pathogen.

When compared with non-invasive tests, the disadvantage of RUT is patients must undergo endoscopy and some patients feel uncomfortable during the procedure. When compared to RUT, ELISA based methods are safe and not influenced by sampling errors.[23] Many commercially available serological kits are widely used for detection of this pathogen. These kits are easy to use and inexpensive. In this study, IgM ELISA yielded 98.4% sensitivity. Sensitivity of both RUT and IgM ELISA was similar across different age groups and gender (Table 3). IgG ELISA yielded 66.4% sensitivity which is significantly lower than RUT and IgM ELISA. Major disadvantage of IgG ELISA is it cannot differentiate current infection from past infection. The IgG antibodies will persist for very long time even after antimicrobial therapy. Therefore, IgG ELISA result remains positive even after the infection subsides.[24,25] Hence, IgG ELISA is useful for epidemiological investigations but not useful for prognostic purpose.[26] The advantage of IgM ELISA over RUT is that it does require endoscopy and the sensitivity & specificity of IgM ELISA is not influenced by gastric atrophy and ulcer bleeding.[27] Therefore, IgM ELISA could be considered as safe and an alternative method for detection of this bacterium.

Many studies suggested a male preponderance in H pylori infection[28,29] and few other studies reported comparable rates.[30,31] One study has reported that female patients with *H. pylori* infection are more vulnerable to develop gastric cancers when compared to male patients.[32] Ibrahim and colleagues[33] searched PubMed and identified 244 population-based studies reporting the prevalence and/or incidence of *H. pylori* infection in both sexes. Among those studies, male sex was associated with a greater prevalence of *H. pylori* infection, both in children (102 studies) and adults (169 studies). Contrariwise, a recent study that assessed Mexican children identified a significantly higher prevalence in girls.[34] Results of our study also suggested that greater prevalence of *H. pylori* infection in female gender compared to male gender. Hence, we believe that further research is needed to understand the mechanisms by which sex may influence the acquisition and/or persistence of infection.

CONCLUSION

In this study, sensitivity of both RUT and IgM ELISA was similar across different age groups and gender. The advantage of IgM ELISA over RUT is that does not require endoscopy. Therefore, IgM ELISA could be considered as safe and an alternative method for detection of this pathogen.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Dr. VRK Women's Medical College Teaching Hospital & Research Center, Hyderabad, India, with reference No: Dr. VRK/2021/027.

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