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METHODS FOR DETECTING HELICOBACTER PYLORI INFECTION: A COMPARATIVE ANALYSIS

Harinder Singh

Research Scholar NIILM University

Dr. R.N. Tyagi

Associate Professor NIILM University

ABSTRACT

The diagnosis of an infection caused by H. pylori can be accomplished through the use of both invasive and non-invasive techniques. A variety of factors, such as the sensitivity and specificity of the tests, the clinical scenario, and the cost-effectiveness of the testing approach, all play a role in determining which diagnostic test is used. The objective of this research was to provide a comprehensive understanding of the interrelationships that exist between the various approaches for diagnosing H. pylori infection and to determine the scope of applications that are associated with each diagnostic technique. Because H. pylori has been linked to a variety of stomach conditions, such as gastritis, gastroduodenal ulcers, and even gastric neoplasia, it is essential to identify the infection as soon as possible and begin treatment in the early stages of the illness in order to stop its progression. Within the context of the pressing requirement to raise the bar for diagnostic criteria that are currently recognised, the purpose of this study is to provide a concise synopsis of both conventional and cutting-edge detection methods that have been successfully utilised to identify H. pylori. A number of criteria are taken into consideration throughout the process of selecting the most appropriate diagnostic strategy. These include determining which test to do based on accessibility, laboratory equipment, and clinical characteristics pertaining to the patient. The objective of this study is to present the many methods that are currently used to diagnose H. pylori, with a focus on both the positive and negative aspects of these methods. Furthermore, the benefits and potential of nanotechnology are examined, as well as the concept of nano(bio)sensors and the development of lab-on-a-chip devices as cutting-edge instruments for the detection, differentiation, and discriminating of H. pylori. There are a number of advantages that are highlighted, such as the fact that these devices are simple to operate, rapid, inexpensive, portable, miniaturised, need a little sample volume, are extremely sensitive, and are selective. Within the framework of intelligent healthcare monitoring systems, it is generally agreed that the development of intelligent sensors will bring about a fundamental transformation in the processes of medical decision-making and acquisition.

KEYWORDS : *Helicobacter pylori , detection methods , H. pylori infection.*

INTRODUCTION

The gram-negative, microaerophilic, spiral-shaped bacteria known as Helicobacter pylori colonise the mucosal lining of the human stomach. At this time, it is believed to be the principal cause of stomach adenocarcinoma, mucosa-associated lymphoid tissue (MALT) lymphoma, gastric ulcers, and gastritis. H. pylori infection is widespread in affluent countries, whereas in less developed countries, the prevalence ranges from 70–90%. Even though there have been recorded cases of oral-oral and fecal-oral infections, the most likely mode of infection is by the transfer of the virus from person to person. The diagnosis of an infection caused by H. pylori can be accomplished through the use of both invasive and non-invasive techniques. Histology, polymerase chain reaction (PCR), microbiological culture, and rapid urease test (RUT) are examples of invasive methods that require endoscopy. Furthermore, these techniques are collectively referred to as biopsy-based testing. Diagnostic procedures that do not involve the use of invasive procedures include the urea breath test (UBT), serology, and stool antigen testing. Test cost-effectiveness, clinical circumstances, sensitivity, and specificity are some of the factors that determine the selection of a particular testing technique. Other factors include the particularity of the test. Importantly, each of these approaches comes with its own set of limitations. Histopathological diagnosis is a method that is frequently employed in countries where endoscopy is performed on a regular basis.

In order to conduct an accurate histological examination, it is necessary to have two key components: biopsies of a high quality and a pathologist with extensive expertise. Incorrect biopsies, factors related to the observer, topographical changes in the stomach, H. pylori density and its uneven distribution, and the type of stain that was used can all lead to the production of false results. The bacterial culture that is acquired from stomach biopsies is considered to be the absolute proof that an individual has an infection with H. pylori. As a result of the more technically difficult nature of the method, the sensitivity of the test and the ability to cultivate may vary from one laboratory to another. With regard to clinical practice, the RUT method is the one that is employed the majority of the time. However, in order to attain a level of sensitivity that is adequate, there must be a significant bacterial load, which is defined as 105 bacteria or more. As a consequence of this, the test is not as recommended for post-eradication follow-up since it is possible that this quantity will not be found four weeks after the medicine that was supposed to eradicate the disease has failed.

In individuals who are not undergoing a gastroscopy, serology is the most basic way for detecting H. pylori infection. This is because it involves the identification of circulating antibodies that are directed against H. pylori. It is unable to differentiate between a current colonisation and an asymptomatic colonisation, as well as between an active H. pylori infection and an infection that occurred in the past.UBT is more sensitive

and specific than other non-invasive tests; nevertheless, its specificity is diminished when other urease-producing bacteria are present in the human gut. UBT is a non-invasive test. In addition, the equipment that is necessary is more advanced and expensive.

The spiral-shaped, gram-negative, micro-aerophilic flagellate bacteria known as Helicobacter pylori (H. Pylori) can be found in the mucosa of the stomach, particularly in the gastric antrum. Approximately fifty percent of the world's population is affected by this bacterial illness, making it one of the most common bacterial diseases in humans. Individuals who test positive for H. pylori have a lifetime prevalence of the condition that ranges from 10–20%, whereas the general population has a lifetime prevalence of 5–10%. The bacteria Helicobacter pylori is the major agent responsible for the development of peptic ulcer disease and chronic gastritis. One of the most important aetiological factors in the development of stomach lymphoma associated with lymphoid tissue (MALT) and gastric cancer has been demonstrated to be the chronic and long-term infection that it causes. As early as October 1994, the International Agency for Research on Cancer (IARC) classified H. pylori infection in humans as a carcinogenic and a confirmed cause of stomach cancer in humans. This classification was based on epidemiological evidence.8. Recent research has established a connection between H. Pylori and illnesses that are not related to the digestive tract, including liver cirrhosis, myocardial infarction, and short stature, in addition to colon cancer. For this reason, it is absolutely necessary that this organism be identified in a timely manner and with a high degree of precision in order to facilitate effective treatment and the avoidance of long-term repercussions. There are two types of diagnostic tests that are now available for the detection of H pylori. These tests are invasive and non-invasive, and depending on the clinical situation, each form of test has advantages and limitations, as well as a relative advantage.
There is no longer a requirement for endoscopic biopsies because of the non-invasive diagnostics, which include stool PCR, urine, saliva, urea breath test (UBT), serology, and faecal antigen test (FAT). Additionally, they have the additional advantages of being speedier, more convenient, and less expensive than other options. They provide a more thorough evaluation of the presence of H. pylori in comparison to invasive tests. This is due to the fact that they are less susceptible to selection error, which is produced by the irregular distribution of H. pylori in the stomach mucosa. They have a lower sensitivity and specificity, which makes them less reliable than invasive procedures. This is because of the difference between the two. Serology, in particular, has been found to have a high sensitivity but a fairly weak specificity, according to recently acquired information.

The Maastricht V Consensus Conference working groups (2017) and the American College of Gastroenterology (ACG) Clinical Guideline (2017) both made recommendations about the diagnostic and treatment options available for H. pylori. Patients with low-grade mucosa-associated lymphoid tissue

(MALT) lymphoma or gastro-duodenal ulcers, patients with a history of recent endoscopic resection of early gastric cancer, patients receiving non-steroidal anti-inflammatory drug therapy, patients with idiopathic thrombocytopenic purpura, patients with anaemia due to iron deficiency without a specific cause, and individuals under the age of 60 who had undiagnosed dyspepsia without alarm features were among those who were included in this group. Tests may be administered to patients who have been taking low-dose aspirin for an extended period of time. When treating juvenile dyspepsia patients, it is recommended that the "test-and-treat" method, which involves the use of non-invasive tests, be preferred over the prescription of proton pump inhibitors or the performance of gastroscopies. Biopsies should be performed on a patient following a gastroscopy in order for the physician to ascertain whether or not the patient's stomach has an H. pylori infection.

A number of strategies are required in order to live in the acidic environment of the stomach.

One of these strategies is the breakdown of urea, which results in the production of ammonia that is poisonous to cells and raises the pH level. This elevates the pH level, which neutralises the acidity and allows bacteria to adhere to and colonise the gastric epithelium. There are a variety of factors that contribute to the increased virulence of H. pylori, and it is well-established that the infection may result in the development of cancer. Lewis b (Leb) and similar terminal fucose residues that are located on blood groups that are expressed on the gastric epithelium were discovered to be bound by the blood-group-antigen-binding adhesin (BabA), according to the observations made. SabA (sialic acid-binding adhesin), AlpA/B (adherence associated lipoprotein A and B), and OipA (outer inflammatory protein A) are some of the other adhesins that play a role in the process of H. pylori attaching to the cell receptors.

The flagellum of H. pylori is an essential component that plays a role in the promotion of chemotaxis, the enhancement of bacterial movement, and the facilitation of the creation of biofilms, which in turn stimulates inflammation and suppresses immunological response.

The virulence factors of H. pylori make colonisation and proliferation easier by shielding the bacterium from the acidic milieu while simultaneously increasing the process of carcinogenesis. In people who possess genetic polymorphisms, such as those found in the IL-1β gene, the alleles IL-1β–511 T and IL-1B–31 C are shown to have a substantial association with an increased likelihood of developing stomach cancer. Among the enzymes, catalase is responsible for inducing mutagenesis, superoxide dismutase is responsible for facilitating colonisation and protecting against reactive oxygen species, arginase is responsible for stimulating apoptosis and preventing the dying of bacteria, and phospholipases are responsible for promoting the breakdown of different lipids and damaging the mucus layer. Phagocytosis is inhibited by the activities of the cytotoxin-associated gene A (cagA) and the type IV secretion system (T4SS). Dendritic cells undergo necrosis and death when they are exposed to gamma-glutamyl transpeptidase. The enzyme

cholesteryl-α-glucosyltransferase has the ability to inhibit phagocytosis and immunological responses. The adhesion of neutrophils to stomach epithelial cells is facilitated by the presence of neutrophil-activating protein.

Even though there are a number of different strains of H. pylori, the only strains that are implicated in this disease are those that have their own unique encoded genes. It has been demonstrated that the cagA gene is associated with ulcer disease, and the vacuolating cytotoxin (vacA) gene is associated with an increased likelihood of developing gastric neoplasia. On the basis of the expression of these genes, it has been determined that there are three distinct groups of H. pylori strains: type I strains, which are extremely virulent, type II strains, which are less virulent, and intermediate strains.

A multitude of risk factors, such as family cleanliness, living in the same house as an infected person, race, ethnicity, cultural customs, and overcrowding in traditional families, all have an impact on the quality of life in nations that are considered to be undeveloped or developing. An relationship between the illness and socioeconomic variables, such as income, education, food quality, and water sources (which is the primary route of transmission) has also been discovered via research on risk factors.

In light of the fact that the illness typically becomes apparent during the early stages of life, preventative measures have to be administered beginning at a young age. The infection incidence does not dramatically decrease despite the fact that living conditions have improved, which highlights the necessity of hygiene and behaviours within the family. According to research, the key elements that contribute to a reduction in the number of diseases that occur are not unsanitary environments but rather awareness and education.

OBJECTIVES

1. To research Helicobacter pylori.
2. To research infections caused by H. pylori.
3. Learning more about histopathology.

METHODOLOGY

Patients as well as examples

The participants in this study consisted of 91 individuals who had upper gastrointestinal endoscopies performed on a routine basis at Firoozgar Hospital, which is a university hospital. These patients had given their consent to take part in the study. The patients ranged in age from 17 to 87 years old, with 39 males and 52 females. The mean age of the patients was 45 years old. Participants who had used proton pump inhibitors, H2-receptor blockers, antibiotic medication, or non-steroidal anti-inflammatory medications

thirty days before to the endoscopy were not allowed to take part in the research. One hundred percent of the patients gave their consent to take part in the research project, which was subsequently authorised by the ethical council of the institution.

A large number of biopsy specimens were taken from the antrum and the corpus. One specimen was reserved for RUT, other specimens were used for histology (both formalin-fixed and paraffin-embedded), and the last specimen was utilised for PCR. Samples of these patients' serum and faeces were collected, and they were kept at a temperature of -20 degrees Celsius until they were required.

Quick urease test

In order to carry out the RUT, a validated test that was not available for commercial sale was utilised. In order to do this test, a solution consisting of 100 milligrammes of urea, one millilitre of distilled water, and one drop of 1% phenol red was prepared by hand. This solution was used immediately before to the endoscopy. A single antral sample was maintained at room temperature and added to the solution while it was immersed. In a period of time that was less than a day, the hue changed from yellow to red, which indicated that the test was successful.

Pathology of the Histopath

Biopsies were collected from the corpus and the antrum for the purpose of histopathology. These samples were then fixed in 10% formalin and forwarded to the laboratory. Many histological sections with a thickness of four millimetres were fixed in paraffin and obtained from each sample. Staining the preparations with hematoxylin and eosin was done, and the Giemsa was evaluated by a number of pathologists who were not aware of the results of the other procedures. Although the presence of H. pylori was confirmed, the strain was not evaluated for its severity.

PCR

The DNeasy Blood & Tissue Kit, which was produced by Qiagen in Hilden, Germany, was utilised in the process of extracting DNA from biopsies utilised for the purpose of the study. Amplification of the ureC (glmM) gene's sequence, which consists of 294 base pairs (bp), was carried out in accordance with the instructions that were presented earlier.It is essential to take note of the fact that the primer pair that was employed in the ureC amplification possesses the nucleotide sequence that is as follows: primers in both directions (forward and reverse). In order to carry out the polymerase chain reaction (PCR), the following parameters were utilised: one cycle of five minutes at 93 degrees Celsius, thirty seconds at 55 degrees Celsius, thirty seconds at 72 degrees Celsius, and one last cycle of ten minutes at 72 degrees Celsius. When

the amplified products were exposed by ultraviolet light, they were visible on agarose gel that contained 2%. The minimum number of times that each test was performed was two.

Stool antigen measurement

Using a polyclonal ELISA stool antigen test (Astra s.r.l., Milan, Italy), stool samples were analysed in accordance with the instructions provided by the manufacturer. In a nutshell, the wells were filled with peroxidase-conjugated polyclonal antibodies as well as diluted samples of faeces. After a period of incubation lasting ninety minutes at room temperature, the sample wells were washed in order to remove any enzyme-labeled antibodies and unbound materials. A spectrophotometric analysis was performed, and the results were read at 450 and 620 nm. For the purpose of determining the levels of H. pylori antigen present in the test samples, a threshold value of 0.2 optical density was utilised. Results were considered negative if the optical density (OD) was less than 0.150. It was determined that samples with optical density (OD) values more than 0.250 were considered positive, whilst samples with OD values ranging from 0.150-0250 were considered to be borderline.

Serology

A blood sample of five millilitres was taken from each patient on the day of the endoscopy, and it was then transported to the laboratory. Separated sera were kept at a temperature of -20 degrees Celsius until the day of the test. In line with the instructions provided by the manufacturer, a serological test for IgG antibodies against Helicobacter pylori was carried out with the use of a commercial Helicobacter pylori IgG ELISA kit (IBL, Hamburg, Germany). Anti-H pylori immunoglobulin (Ig) G titers were categorised as positive if they were more than 12 U/ml, negative if they were lower than 8 U/ml, and ambiguous if they were between 8 and 12 U/ml. Positive titers were regarded to be specific for the presence of H pylori.

RESULT

The findings of the positive and negative histology were acquired within a short period of time. A few minutes to a day was all that was required to see the outcomes of the RUT. A successful polymerase chain reaction (PCR) was evidenced by the presence of a band on an agarose gel that measured 294 base pairs (Fig. 1). Due to the fact that our research did not involve the cultivation of H. pylori from biopsies, other endoscopic-based procedures, including as RUT, PCR, and histological staining of the biopsies, were considered to be the gold standard for assessing the specificity and sensitivity of each test. If the results of two out of three tests were positive, then the patient was considered to have an infection caused by H. pylori. According to the criteria that were provided, 45 of the patients (49.5%) were determined to be free of H. pylori infection, whereas 46 of the patients (50.5%) were found to have the infection.

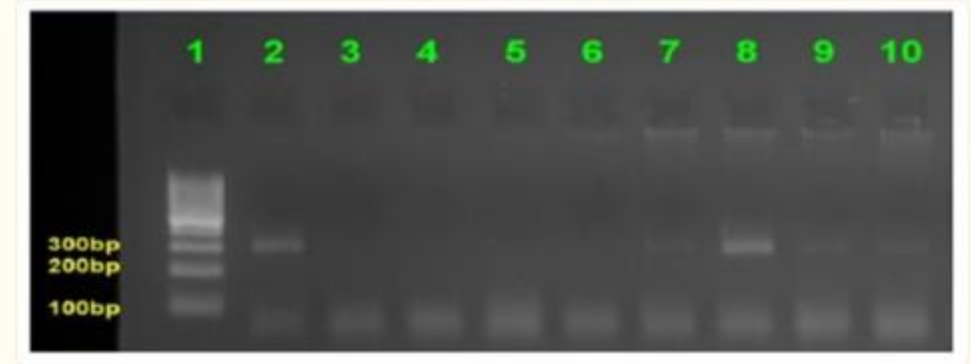

Fig. 1 PCR results for H. pylori using primers based on the glmM gene. Lanes 1–10 include patient biopsy samples; Lanes 1–3 contain positive and negative controls; and Lane 4–10 contain ladders.

The calculated sensitivity, specificity, predictive values, and accuracy of the ELISA- and biopsy-based diagnostic tests for all 91 patients are presented in Table 1. These results are compared to the gold standard.

Table 1 A comparison of five distinct approaches to the gold standard for diagnosing H. pylori infections.

Methods		**kola stomata**		**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	Accuracy **(%)**
		Positive	**Negative**					
	Positive	**44**	**10**					
Histology				95.6	77.8	**81.5**	**94.6**	86.8
	Negative	**2**	**35**					
	Positive	**44**	**0**					
RUT				95.6	**100**	**100**	95.7	97.8
	Negative	2	45					
	Positive	43	2					
PCR				93.5	**95.6**	**95.6**	93.5	94.5
	Negative	3	43					
	Positive	42	20					
Serology				91.3	**55.6**	**67.7**	86.2	73.6

	Negative	4	25					
	Positive	34	6					
Stool antigen				73.9	**86.7**	**85**	76.5	80.2
	Negative	12	39					
Total		46	45					

CONCLUSION

Any method that can detect H. pylori infections in a short amount of time and at a reasonable cost would be great. The identification of an H. pylori infection can be accomplished by a variety of methods. In the context of normal clinical diagnostics, the urease test, the histological examination, the urea breath test, serology, bacterial culture, and the stool antigen test are all helpful methods for determining whether or not an individual is positive for H. pylori infection.Histopathology is still widely used as the primary diagnostic approach in cases where persons with upper gastrointestinal symptoms are suspected of having the condition or in areas where the condition is particularly prevalent. At one point in time, histopathology was considered to be the initial diagnostic process for the diagnosis of H. pylori. A reliable and precise histological diagnosis of H. pylori gastritis has a substantial influence on clinical treatment since it serves as a therapeutic indicator. On the other hand, a lot of past investigations have demonstrated high inter-observer variation, which suggests that the pathologist's capabilities to diagnose H. pylori histopathologically are rather satisfactory. A pathologist would frequently discover more positive results in the present study, even when the other tests returned negative results. This demonstrates that the experience and expertise of a pathologist do have an effect on the specificity and sensitivity of the histological examination.
In the current experiment, the sensitivity of the rapid urease test was 95.6%, which is pretty comparable to the findings that were obtained by the authors who came before. In addition, the specificity of RUT is equivalent to that of the findings of other researchers, despite the fact that, in contrast to the findings of prior studies, we did not receive any false-positive results from RUT.Molecular approaches provide a number of benefits, two of which are their speed and their ability to minimise the influence of transit conditions. There have been a great number of PCR methods developed up to this point in order to directly identify the organism that is present in clinical samples. There are a great number of genes that have been used as targets, such as the 26-kDa species-specific antigen (SSA) gene, the ureA gene, the ureC (glmM) gene, the 16S rRNA gene, and the cagA gene. During the ureC amplifications, Lage and his colleagues proved that H. pylori was the only urease-positive or related bacteria that generated the amplified DNA products that were

predicted. Our analysis demonstrates that the ureC (glmM) primers are capable of validating the sensitivity and specificity of the PCR test.

There is a wide variety of serological tests that are available for commercial use. The fact that they are inexpensive and easy to use contributes to their widespread adoption. However, tests that rely on the detection of specific antibodies are not useful in detecting whether or not H. pylori has been completely eradicated. This is due to the fact that antibody titers can remain at high levels for a number of months after the infection has been removed. The serology test achieved the lowest levels of specificity and accuracy when compared to the other tests that were used in this analysis. IgG serological tests often have a poor level of accuracy since it is difficult for them to differentiate between infections that occurred in the past and those that are occurring at the present time. However, due to the fact that virtually all persons who had previously received treatment were excluded from consideration on the basis of the questionnaire, and because H. Pylori infection did not always cure on its own, a single positive serology test result may suggest either a false positive or a previous infection.

The stool antigen tests that are currently available for purchase include a variety of different types. Despite the fact that the sensitivity and specificity rates that are given might vary, the majority of the studies have demonstrated that the stool antigen test is beneficial for both the initial diagnosis of H. pylori infection and the follow-up tests that are performed following treatment. Premier Platinum HpSA, the first and most frequently used valid H. pylori stool antigen test, has been indicated as a reliable alternative to UBT in the first diagnosis and follow-up phase.

This is despite the fact that there are certain studies that indicate a lesser degree of accuracy using this test. Within the scope of our analysis, the stool antigen test exhibited the lowest sensitivity (73.9%), as well as the lowest specificity score (86.7%). Throughout the course of our investigation, we were only able to identify one research study that had employed this particular kit. In spite of the fact that they were conducted under almost identical conditions, their findings (85% for sensitivity and 90% for specificity) were superior to those of our study. There is a possibility that the accuracy of the test will change from one batch to the next, and Makristhati et al. have already established the possibility of intertest variability. Intertest variability and methodological mistakes are two possible explanations for these disparities, which might be attributed to the fact that they exist.On the basis of the findings of the study, the following is a ranking of the accuracy of the tests for identifying H. pylori: stool antigen test > serology > PCR > histology > RUT. However, there is a possibility that the order would be somewhat different in trials that are equivalent to this one. In general, however, the data suggest that approaches based on biopsy are preferred above other techniques in almost all studies; none of these techniques can be considered the gold standard on their own

at this time. In order to confirm an H. pylori infection, it is recommended that non-invasive and biopsy-based procedures be utilised simultaneously within the diagnostic process.

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