Review Research

OVERVIEW OF LABORATORY DIAGNOSIS OF HUMAN HELICOBACTERIOSIS

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ABSTRACT

Helicobacter pylori (H.pylori)(HP) is the causative agent of helicobacteriosis. Found often there, it irritates and inflames the stomach and small intestinal walls. In 2015, an estimated 4.4 billion people globally carried *Helicobacter pylori* infections. Adenocarcinoma risk has increased since this bacterium was originally isolated, suggesting that it may be one of the most prevalent human bacterial infections. As a result, the World Health Organization (WHO) designated it a Category 1 carcinogen in 1994. Several assays are available for use in the laboratory for the diagnosis of *H. pylori* infection from patient samples. These tests may be broken down into two categories: invasive and non-invasive treatments, each with its own set of benefits and drawbacks. However, there are limitations to each in terms of actual clinical use. Clinical context, probability ratio of positive and negative tests, cost-effectiveness of testing approach, and availability of the test should all factor into the choice of test. Paying close attention to the positive news about *Helicobacter pylori* is crucial to reducing the incidence of *H. pylori* infection and its associated symptoms. These problems can be avoided altogether if the illness is caught and treated in time. In this review, we will discuss the methods used to identify Helicobacteriosis in humans in the laboratory, both invasive and non-invasive.

Keywords: Advantages, disadvantages, *Helicobacter pylori*, invasive, laboratory diagnosis, non-invasive.

Introduction

Helicobacter pylori (*H.pylori*)(HP) is the causative agent of helicobacteriosis. often found in the digestive tract, and may irritate the stomach and intestinal linings (Hooi et al., 2017; Ferlay et,2019; Bashir and Khan, 2023). There were 4.4 billion people infected with *H. pylori* in 2015, making it one of the most widespread human bacterial diseases worldwide (Almashhadany and Mayass, 2017; Lanas and Chan, 2017; Almashhadany et al., 2023). Gastritis, ulcers, and cancer of the stomach are all caused by *H. pylori*, a category 1 carcinogen. Roughly half the global population has been infected. *H. pylori* infection risk factors include low income, unhealthy habits, and a poor diet (Alexander et al., 2021; Almashhadany et al., 2022; Reyes, 2023)

Several authors compiled a list of conditions for which clinical guidelines suggest testing for Helicobacteriosis. 1- Peptic ulcer in the family history; 2- first-degree relative with stomach cancer; 3- Having a family member who is currently infected with *H. pylori*; 4- Iron deficiency anemia (IDA) for no apparent reason; 5- Idiopathic Thrombocytopenic purpura of unknown cause (ITP); 6- Lack of vitamin B12; 7- Peptic ulcer disease, either present or in the past; 8- Chronic dyspepsia; 9- Use of aspirin or other non-steroidal anti-inflammatory medicines (NSAIDs) over an extended period; 10 - Gastric precancerous lesions; 11- Intestinal adenocarcinoma; 12- Mucosa-associated lymphoid tissue (MALT) lymphoma (Ma et al.,2016; Chey et al., 2017; Mahachai et al., 2018; Malfertheiner et al., 2022).

There are several assays available for use in today's laboratories for the diagnosis of *H. pylori* infection from patient samples. Invasive and non-invasive methods with varying degrees of sensitivity and specificity may be used to conduct these tests, respectively. However, there are restrictions on how each may be used in practice. Clinical context, probability ratio of positive and negative tests, cost-effectiveness of testing method, and availability of tests should all factor into decision-making about which tests to utilize (Tiwari et al., 2005; Nguyen, 2009; Trevethan, 2017; Alfaro et al.,2023). Patient preference, the availability of diagnostic assays, and the sensitivity and specificity of the assay are just a few of the factors that go into deciding which diagnostic approach to choose.

Invasive Methods

Endoscopic procedures are required for these examinations, which may be distressing for patients. A biopsy sample of the upper stomach mucosa is analyzed, and the microorganism is identified. Additionally, there will likely be erroneous negative outcomes (Lee and Kim, 2015; Park and Lee,2019; Urgessa et al.,2023). However, a gastroenterologist may take biopsies (small tissue samples) of the stomach lining during the procedure of an upper endoscopy. Afterward, the biopsies are analyzed in various ways.

Rapid Urease Test (RUT)

The biopsy sample is submerged in a solution designed to identify the presence of H. pylori's urease enzyme. The presence of H. pylori is indicated by a positive result because urea in the solution is converted to ammonia by the urease. Specificity is between 95% and 100%, whereas sensitivity is

between 85% and 95% (Wang et al.,2015). RUT is advantageous since it is a cheap, simple, quick, and generally accessible test that can certify a patient's recovery. There are several factors, such as the density of bacteria in the biopsy, the use of Proton Pump Inhibitors PPI, antibiotics, bismuth, H2 receptor antagonists, the presence of blood, or reading the test earlier than recommended, that could lead to false negative results, and endoscopy is required. Reduced precision following antibiotic or proton pump inhibitor use (Kinoshita et al, 2018; Gong and Zhang,2023).

Histological evaluation

H. pylori bacteria in the stomach lining may be identified by staining a biopsy sample and seeing it under a microscope. The traditional "gold standard" method for *H.pylori* infection detection has been histological assessment (Orhan et al.,2008; Gong et al.,2021). The sensitivity and specificity of this assay are high, it is documented as 95% and 99%, respectively (Calvet et al., 2009). Gastritis histological staging (gastric atrophy and intestinal metaplasia) may be determined using this approach, which is useful for ranking the patient's risk of developing cancer. Site, number of biopsies, and Proton Pump Inhibitors are just a few of the variables that might affect the reliability of a diagnosis, which is one of the many drawbacks. Patients on proton pump inhibitors (PPIs), those who are taking antibiotics, and those who have expertise in pathology (Nguyen, 2009; Fossmark et al., ,2019).

Culture

The "gold standard" for the identification of H.pylori infections is the microbiological isolation of the pathogen. Cultures have been retrieved from a wide variety of sources, including stomach biopsies, feces, vomit, aspirate, brushings, dental plaque, and saliva (Ndip et al.,2003). To grow the organism, however, the stomach mucosal biopsy material is now regarded as the best and simplest source. Whether using a solid or liquid medium, this technique may be used to selectively grow *H. pylori*. One example is the use of Columbia blood agar medium supplemented with Skirrow's antibiotics (10mg Vancomycin, 2500 units Polymyxin B, 5mg Trimethoprim) and 7% defibrinated horse blood (Mégraud and Lehours ,2007; Malfertheiner et al 2017).

Colony morphology, gram staining, and a positive response to oxidase, catalase, and urease tests all contribute to the identification of the bacteria (Nguyen, 2009). The time between sample and culture, transit temperature, transport medium, and time spent exposed to air are all factors that may impact how long *H.pylori* survives (Yuen et al.,2005). This method, on the other hand, permits the collection of stomach mucosal samples at the time of biopsy for use in subsequent research, including culture, histology, the fast urease test, and molecular methods.

The primary benefit of this procedure is that it gives the antibiotic sensitivity of *H. pylori*, but it is not very accessible since it is not conducted in all hospitals and it is costly and tedious. After one or more treatment lines have failed, knowing which antibiotics will be effective may assist guide particular eradication programs. Antibiotic resistance may be detected by culture in individuals receiving several

therapeutic failures. Antibiotic resistance may also be detected at the population level using this method (Llor and Bjerrum,2014; Mahachai et al., 2018; Tshibangu-Kabamba and Yamaoka,2021).

The stomach biopsy with subsequent *H.pylori* culture has the best specificity (almost 100%), but the lowest sensitivity (85%-95%) due to the significant complexity of transporting and cultivating *H.pylori* (Nguyen,2009; Urgessa et al.,2023). The culture approach has the greatest specificity, can determine antimicrobial susceptibility, and demonstrates cure (often several days), sensitivity to PPI and antibiotic usage, and difficulty in transferring samples and cultivating H. pylori (Šeligová et al., 2020; Cardos et al., 2022; Francesco et al., 2022).

Polymerase Chain Reaction (PCR)

Using this method, even trace quantities of *H.pylori* DNA (genetic material) in biopsy samples may be amplified and identified. The sensitivity and specificity of PCR for detecting H.pylori in stomach biopsies, gastric juice, saliva, and stool samples are all quite high (Schabereiter-Gurtner et al., 2004; Pichon et al., 2020). The sensitivity of PCR was 100% for stomach biopsy and 98% for stool samples, while the specificity was also quite good at 98% (Mackay et al., 2003; Frenck et al., 2006; Ricci et al.,2007). The sensitivity of *H.pylori* to certain antibiotics may be determined, and the PCR approach can be used on a wide variety of materials, including gastric biopsy specimens, gastric juice, saliva, and feces, without the need for any specialized processing supplies or transportation. On the flip side, molecular techniques can be costly, positive results may not indicate active infection because they can also detect the DNA of dead organisms, and the technology is still in its infancy (Mackay et al, 2003; Frenck et al, 2006; Ricci et al, 2007; Mishra et al.,2008). Unfortunately, it is still under development and only works effectively with stomach samples, thus it is not widely available.

Non-invasive Method

Diagnosing *H.pylori* infections using non-invasive diagnostics is preferable and just as accurate. They save patients the trouble and discomfort of having an endoscopy (Lin et al, 2004; Abadi, 2018). Proxy measures of infections, such as these, offer certain benefits, including low cost, low risk to the patient, low difficulty, and high speed, and although they are useful in detecting possible infections or illnesses, they may not always give a definite diagnosis. Non-invasive test findings should be confirmed by more definitive techniques, such as culture or histopathology (Calik et al,2016; Al-mashhadany,2018). Because gastroscopy is an intrusive procedure, it carries with it the risk of complications from the anesthesia or the procedure itself. However, endoscopy may be contraindicated (a particular scenario in which a medicine or operation should not be used because of the risk it poses to the patient) in people at risk or with comorbidities (the existence of more than one illness inside the human body at the same time). Furthermore, gastroscopy can only be done in hospitals, making it more difficult to access. Non-invasive testing has been developed as a solution to these issues (Nguyen,2009; Urgessa et al, 2023).

Stool Antigen Test

The detection of H. pylori infection using stool antigen testing is both reliable and accurate. H. pylori antigens may be detected in a stool sample using this test. Large proteins found on the surface of pathogens including viruses, bacteria, fungi, and other foreign particles are called antigens. The presence of *H.pylori* in stool samples may be reliably detected using the stool antigen test (Gisbert et al., 2006; Almashhadany and Mayass,2018 ;Alfau et al., 2023). Polyclonal antibodies, which are a collection of antibodies made by various B cells in response to an antigen, were used in the first kits to detect antigens. Recently, tests using monoclonal antibodies (mAbs)—antibodies made from a single B cell clone that each identify a unique epitope on the antigen—have been created; these tests are more precise. The Stool Antigen Test (SAT) may be performed in two distinct ways, one using fast immunochromatography assay (ICA) and the other using enzyme immunoassay (EIA). Although EIA test kits are more widely available and may be used by both primary care providers and patients, they fall short of the ICA in terms of sensitivity and specificity (Nguyen,2009; Mahachai et al., 2018; Al-Mashhadany,2020; Urgessa et al., 2023).

The pooled sensitivity of the monoclonal stool antigen test was higher (94% vs. 83%) and the specificity was similar (97% vs. 96%) to that of the polyclonal stool antigen test. So, monoclonal stool antigen testing is useful not only for diagnosis but also for confirming eradication (Resina et al., 2021). SAT has the potential for diagnostic and post-eradication confirmation, is inexpensive, and is simple to implement. The EIA-based approach has high sensitivity and specificity and is widely accessible; primary care physicians (PCPs) or the patient themself may choose to utilize it.

The EIA-based technique has the limitation of requiring laboratory analysis of the material, while the ICA-based method has the drawback of having poor sensitivity and specificity. The stool antigen test becomes negative again anywhere from 5 days to a few months after the pathogen has been eradicated, making SAT a helpful technique for verifying eradication (Silva et al, 2010 ; Almashhadany et al., 2018).

Serological Tests

Blood tests, or serological testing, are non-invasive and may be used to track *H.pylori* antibody levels in the body. Antibodies are created as part of the immune response when a person is exposed to H. pylori. This method can determine whether a person has been exposed to *H.pylori*, but it cannot tell if they are now infected or have been infected in the past (Narayanan et al., 2018; Sabbagh et al., 2019). Circulating IgG antibodies can be detected by Enzyme-Linked Immunosorbent Assay (ELISA) or latex agglutination (Luzza et al.,2000; Quach et al., 2014; Zaman et al., 2020).

The sensitivity and specificity of the assay were 85- 92% and 79 – 83% respectively (Zambon et al., 2004). Serological tests have their uses, and they are reliable in some scenarios. These scenarios include bleeding peptic ulcers, stomach mucosal atrophy, and the use of antibiotics or Proton Pump Inhibitors (PPIs) in the recent past. The main problem with serology is that tests can be positive many

months after eradication because antibodies remain in the blood for a very long period, so it is not useful for post-eradication confirmation and commercial tests need to be locally validated. A previous exposure to *H.pylori* is all that is indicated by a positive serological test. Infections, both ongoing and cured in the past, are treated identically. As a result, it may not be the most reliable test for identifying a current illness. Additional testing to confirm infection may be necessary if a serological test returns a positive result, especially if the patient is experiencing symptoms or is at high risk for developing problems associated with *H.pylori*.

Antibody testing using saliva and urine is less prevalent but needs particular consideration. Tests based on urine were only moderately sensitive (84.7%), specific (89.9%), and accurate (87%) overall. Despite having a reasonable sensitivity (81%) and specificity (73%), findings from salivary testing were disappointing (Stefano et al.,2018).

Urea Breath Test (UBT)

Non-invasive diagnostic testing for *H.pylori* infection has been mostly replaced by the Urea Breath Test (UBT) (Aumpan and Mahachai,2023). Antibiotics and proton pump inhibitors are two examples of pharmaceuticals that might skew test results and should be avoided for a period determined by the patient's healthcare professional before the test. The reliability of the test findings depends on the patient abstaining from food and drink for a certain period (often 6-8 hours).

The test requires the patient to consume a unique liquid containing the organic component urea. A trace quantity of radioactive or non-radioactive carbon may also be present in this fluid. In the presence of *H.pylori*, the urea in the liquid is broken down into carbon dioxide (CO2) and ammonia by the bacteria in the stomach. Depending on the version of the test being conducted, the carbon dioxide may or may not be radioactive. The patient produces a breath sample by exhaling it into a collecting bag or specialized container after a certain amount of time, often between 30 minutes and an hour after ingesting the urea solution. Carbon dioxide levels are determined by analysis of the breath sample. Increased breath carbon dioxide may be traced back to the presence of *H.pylori* in the stomach, which breaks down urea. Professional medical personnel can ascertain the presence or absence of *H.pylori* based on the quantity of carbon dioxide measured. A live *H.pylori* infection is indicated by a positive test result. With a sensitivity and specificity of 93% and 92%, respectively, UBT may still be utilized after therapy to confirm the eradication of H. pylori in patients with upper gastrointestinal bleeding. UBT has great sensitivity and specificity and may confirm a patient's recovery with little to no out-of-pocket expense. It is still widely used as a diagnostic tool for *H.pylori* infection both before and after eradication therapy. Patients using PPIs or antibiotics before to a UBT should know that doing so will negatively impact test results (Ferwana et al., 2015).

Conclusion

Since *H.pylori* is such a common and troublesome infection, our review has led us to the conclusion that it deserves a lot of attention. In 2015, 4.4 billion people were afflicted with the bacteria. Infection of the stomach lining is widespread, and it may lead to conditions including gastritis and peptic ulcers. Several papers over the past few decades have linked *H.pylori* infection with extra gastrointestinal complications such as metabolic syndrome, diabetes mellitus, cardiovascular disease, neurodegenerative illness, hematological disorders, ischaemic heart disease, fatty liver disease not caused by alcohol, and allergies. Therefore, early diagnosis, proper monitoring, and further therapy options are required. Early diagnosis and effective eradication of the infection are essential for reducing or eliminating Helicobacteriosis and improving H. pylori treatment. This can be achieved through the use of periodic effective tests and retesting consistently, meticulous endoscopic observation frequently, and health promotion.

Conflicts of Interest

The authors declare no conflicts of interest.

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