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A COMPARATIVE STUDY OF DIAGNOSIS METHODS OF *HELICOBACTER PYLORI* IN PATIENTS WITH GASTROENTERITIS IN BASRAH PROVINCE

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ABSTRACT : *Helicobacter pylori* affects more than half of the world's population and causes many diseases in humans, such as gastritis, duodenum and peptic ulceration, which may develop into gastric cancer and cancer of the Mucosa-associated lymphoid-tissue lymphoma. The treatment of these bacteria depends on their accurate diagnosis. The aims of this study are to compare the methods that used to detect *H. pylori* bacteria and to determine the most accurate, fastest and least expensive diagnostic method. A total of one hundred samples were collected from patients attending the endoscopy unit. The samples divided into tissue biopsy, stool and blood samples from each patient were used to detect IgG antibodies formed against *H. pylori* infection, fecal samples for rapid fecal antigen detection (Step). Also, the tissue biopsy was used for bacterial transplantation and rapid urease enzyme testing. Invasive methods were compared to non-invasive to choose the most efficient method. Rapid fecal antigen testing in non-invasive methods were found to be improved in diagnosis more than in invasive methods. For the invasive methods, the rapid urease test to be the best.

Key words : *Helicobacter pylori*, invasive tests, noninvasive tests.

INTRODUCTION

The Australian scientists Warren Robin and Barry Marshall discovered *Helicobacter pylori* in 1982 and explain its role in causing most cases of peptic ulceration and gastritis (Marshall and Warren, 1984). This bacterium is one of the important pathogens of the human being as it causes many stomach infections (Asma'a Yahya Erzooki, 2016). These bacteria are characterized when they are in their colonies become convex, they are transparent. similar to the drop of water, when are not spore and non-decomposition of blood. They also characterized by the phenomenon of polymorphism that may appear in the form of a seagull wing as well as in the form of bacilli and in the old culture appear in the form of spheres and is moving by its unipolar monolithic whips (Al Sulami *et al*, 2010). *H. pylori* is a low-volume microaerophilic bacterium that is negative for gram- stain and helical shape (Smyk *et al*, 2014). The bacterium has a several major virulence factors, including the gene associated with cytotoxin A. (CagA), the cellular toxin that forms the vacuolizing (V ac A), as well as its production of the urease enzyme that analyzes the urea in the middle to the ammonia that has the effect. antacid around in the lining of the stomach (Bakir, 2018). The prevalence of *H. pylori* is also associated with living conditions and socio-economic conditions of the family (Sugimoto *et al*, 2009). Several epidemiological studies indicate that more than half of the world's population is infected with *H. pylori* (Karczewska *et al*, 2012). But the prevalence of bacterial infection varies between countries. In developing countries, for example, the prevalence of *H. pylori* infection is more than 70% (Stasi and Provan, 2008). Some epidemiological studies indicate that the infection rate is equal between males and females, but other studies have shown that the infection rate is higher in males than in females (Bennett *et al*, 2019). More than 81% of people with *H. pylori* showed no signs of disease (Markovska *et al*, 2011). However, several studies have shown the relationship of *H. pylori* bacteria to several diseases that affect the digestive system that these diseases are characterized by many different symptoms, the most important of which are: dyspepsia and acidity, malena, vomiting, loss of weight and abdominal pain. These symptoms vary in severity from one person to another, depending on the person himself (Boyanova, 2008). *H. pylori* infection may be associated with other diseases occurring outside the stomach such as Diabetes Mellitus, cardiovascular diseases, dyslipidemia, and reproductive disorders (Pellicano *et al*, 2000; Perez *et al*, 2004).

MATERIALS AND METHODS

Collection and inoculation of samples

The study included the collection of one hundred samples from patients in different ages ranged from (4-75 years). Two biopsies were collected from each patient and the first biopsy was placed in sterile plastic tubes containing (5) ml of Normal Saline 0.9% to preserve the tissue biopsy until it was implanted in the center of the hard Chocolate Agar. The second biopsy from the patient was used to test urease production and the tubes were kept in a refrigerated box containing ice to transport the biopsy to the laboratory within two hours. Also, 100 blood samples and 63 stool samples were collected from the same patients. It was not possible to collect a sufficient number of stool samples equal to the total number of samples because the patients did not eat for the purpose of the endoscopic examination as directed by the specialist. The breath test used to determine the helical bacteria was based on 10 samples only for patients not attending the private laboratory.

Methods Requiring Endoscopy

1. Rapid urease testing

To perform this test, a tissue biopsy removed from the stomach antrum is placed in a liquid urea medium containing a red phenol reagent (pH index) then incubated at 37°C for 24 hours. The urea will be decomposed into ammonia and carbon dioxide, and as a result the pH of the medium will increase and the color will change from yellow to pink (Lopes *et al*, 2014).

2. Bacterial agriculture

Parsonite method was used to transplant the samples (Chen *et al*, 2012). After arrived the samples to the laboratory should not exceed two hours from the moment of collection until the transplantation, placed on a sterile glass slide and cut each into small parts using sterile scalpel and mix well. (Loop) was published on the selective medium Chocolate Agar Streaking, then transferred the dishes to the anaerobic container (Anaerobic Jar) and put gas release kit (Gas generating kit) inside the anaerobic container after activated by adding 10ml of distilled water to the contents of the kit. Then we sealed the container tightly for the purpose of providing gas conditions 5% oxygen, 85% nitrogen, 10% carbon dioxide, anaerobic vessel was placed in the incubator at 37°C for 7-14 days, after the incubation period, diagnosis of bacteria by biochemical tests (Gram-stain, Oxidase Test, Catalase Test, Urease Test, Motion Detection by Optical Microscopy) and other diagnostic methods (Holt, 1994).

Non–endoscopic methods

1. Serological test

Using a rapid, one-step qualitative immunological test to give a preliminary diagnosis of these bacteria, the IGg antibodies of *H. pylori* in serum, taken from all samples in the study, were investigated according to the instructions of the equipped company (Biozek). It contains a hole where (4-3) drops of serum if this serum contains the antibodies IGg formed against bacteria interacts with the antigen molecules encapsulated by the surface of the hole, this reaction leads to change the color of the line that appears in the examination area, which shows the test positive, While the absence of the red line in this area indicates negative. The test calculated within 10 minutes and then neglected the result (Chen *et al*, 2002).

2. Stool Antigen Test (SAT)

This test is used to investigate the antigens produced by *H. pylori* in the stool by taking stool samples from the patient and tested using several ready diagnostic kits according to the Spanish manufacturer (Certest Biotec) instructions. The stool is diluted by adding part of the sample to closed tubes containing on Buffer and shake well, after one minute add 4-3 drops of diluted sample to the room (S) and wait for the result to appear within 10 minutes, but after this period is considered wrong (Al – Mahdawy, 2013).

3. Breath test

The basis of this test is based on the ability of *H. pylori* bacteria to produce urase enzyme. Patients are given urea with radioactive carbon ^{13}C or ^{14}C orally in solid form Tablets or liquid. In the presence of *H. pylori* in the stomach, these bacteria analyze urea by enzyme the urease to ammonia (NH_3) and carbon dioxide (CO_2), which is excreted from the body through breathing (exhalation), contains radioactive carbon and can be measured with special devices (Marshall *et al*, 2008).

RESULTS

The results of the present study showed the possibility of isolating and diagnosing *H. pylori* infection from patients using different methods and samples. The bacterium *H. pylori* was isolated and diagnosed from biopsy tissue biopsy, which was removed from the Antrum area and blood and stool samples were obtained. Methods used to isolate and diagnose these bacteria include, Bacterial Culture Tissue biopsy samples were planted on chocolate agar medium and *H. pylori* was isolated from 61 samples out of 100 samples, whereas the bacteria did not appear in 39 samples, or 61% isolation rate. The bacteria were diagnosed based on the shape of the

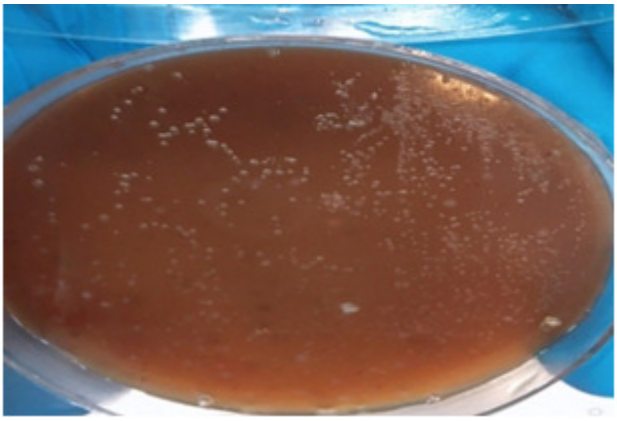

Fig. 1 : *H. pylori* colonies developing in Chocolate Agar medium.

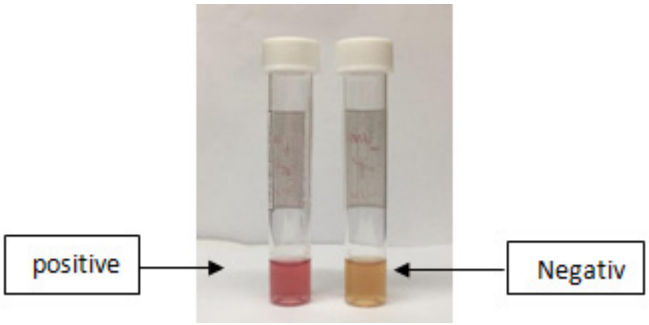

Fig. 2 : Illustrates the urease test for *H. pylori* bacteria.

Fig. 3 : shows the blood test.

growing colonies on the medium, where a very small convex circular edges appeared transparent and similar to water droplet shape or gray as in Fig. 1.

Biochemical tests were used to diagnose *H. pylori* bacteria, which was positive for oxidase and catalase tests and also gave a positive result for urease test. This test depends on the ability of *H. pylori* bacteria to produce the urease enzyme. From yellow to pink, microscopic examination of movement test, and use of Gram Stain as it appeared in the form of helical cells or bent bending and appeared negative for Gram stain. Rapid urease testing, this test is a reliable test for the detection of *H. pylori* bacteria, which gave a positive result in 91 samples out of 100 samples, while 9 samples had a negative result as in Fig. 2.

Serological test : This tests was carried out to detect the antibodies formed against *H. pylori* and gave positive results in 85 out of 100 samples and negative results of 15 samples as shown in Fig. 3.

Stool antigen test this test was used to detect *H. pylori* antigens. In this study, 59 cases were positive and 4 cases were negative out of 63 cases, the percentage of positive cases was 93.6%, which is the highest among the methods used in the present study. The test can be illustrated in Fig. 4.

Breath test Urea, this test was carried out to detect the germ of the stomach and gave a positive result in 9 out of 10 samples, while a negative result was recorded in one case only. Table 1 shows the methods used to isolate and diagnose *H. pylori* bacteria and the percentage of positive and negative cases for each method. The statistical analysis results showed a significant difference between the screening methods used in the current study.

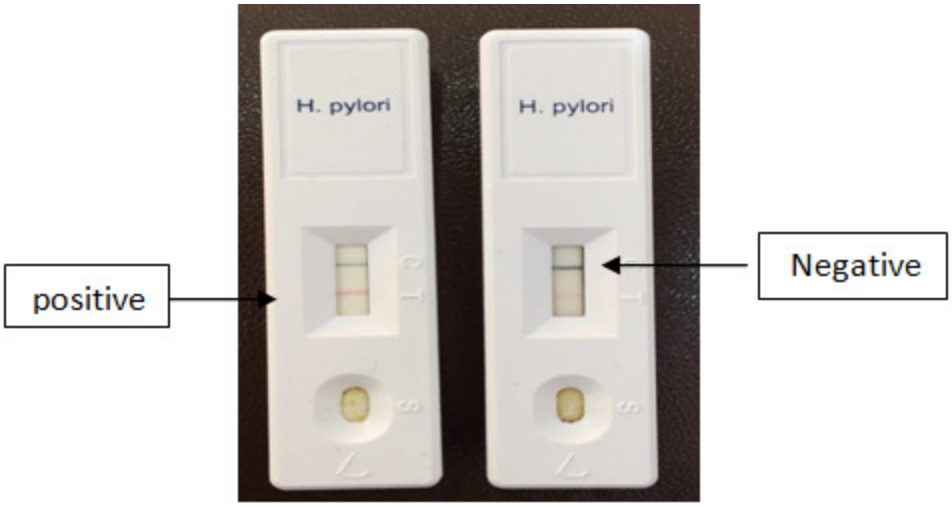

Fig. 4 : Illustrates the fecal antigen test for the detection of *H. pylori*.

Table 1 : Comparison of ratios between methods used to isolate *H. pylori.*

Methods used	Number of samples	% Number of positive cases(%)	Number of negative cases(%)
Urease (Biopsy)	100	91(91%)	9(9%)
Strip (serum)	100	85(85%)	15(15%)
Stool Rapid test	63	59(93.6%)	4(6.3%)
Bacterial cultural Biopsy	100	61(61%)	39(39%)
Urea breath test	10	9(90%)	1(10%)

$P < 0.05$, $\chi^2 = 53.730$

DISCUSSION

Several studies have referred to the epidemiological role played by *H. pylori* bacteria, which is one of the leading causes of gastritis and is associated with gastric ulcer, duodenal ulcer, stomach lymphoma and stomach cancer (Hwang *et al*, 2019; Kountouras *et al*, 2019; Paik *et al*, 2019).

In this study, *H. pylori* was isolated and diagnosed from gastric biopsy using the culture method on the medium of solid chocolate agar, the isolation rate to be 61%.

Other studies conducted in Basra governorate showed a variation in the *H. pylori* isolation rates using tissue culture transplantation method, some of which agreed with our results and others are not as follows: 62% (AL-Abdula *et al*, 2013), 71% (Khudor, 2007), 68% (Al-Sulami *et al*, 2012), 71% (Bakir *at al*, 2004). Also, studies in different countries showed that the *H. pylori* infection incidence was different and disagreed with our results 35.2% (Milani *et al*, 2019), 39.21% (Sullivan *et al*, 2019). This results showed that the bacterial transplantation test recorded the lowest isolation rate compared to other methods used to detect this bacterium, may be due to the nature of the sensitive growth requirements of these bacteria, or because of the small size of the sample, or the patient taking antibiotics and proton pump inhibitor (PPIs), which is taken by patients before taking the sample, leading to a decrease in the number of bacteria in the biopsy (Chen *et al*, 2012). Also, our results showed that the percentage of positive samples for the rapid urease test to be 91% and similar to the results of 98.4% (Hussein *et al*, 2016), 86% (Bakka *et al*, 2002). In the other hand, this results disagreed with the results of Al-Jumaily (2013) to be (44.56%). This may be due to the error in the place of sampling, the size of the sample is insufficient and the low density of *H. pylori* leading to give false negative results, and can be recorded false positive results for several reasons, including the presence of other bacterial species capable of secreting urease enzyme, as well as increase the duration the lap time is about 24 hours. The study showed positive test for the detection of antibodies to *H. pylori* in 85% of the samples. This results are in line to those obtained by Khalifehgholi *et al* (2013) to be (91%) of the infected persons were positive for serological test. Our results were not consistent with Luo (2015), which received a 55.6% serological test. The differences results may be due to the diversity of the immune response from one person to another, Antigenic mixing can also occur

Table 2 : Evaluation of Positive and Negative Characteristics of Diagnostic Tests for H. pylori

Endoscopic Testing	Advantages	Disadvantages
Culture	Excellent diagnostic method, determines the antibiotics sensitivity.	Testing is expensive and complex and requires time and special circumstances, requires the use of periscope to obtain samples.
Rapid urease test	The test is quick, simple, and less expensive than the transplant method.	May give false results,, requires the use of periscope.
Nonendoscopic Testing	**Advantages**	**Disadvantages**
Serology	Available, inexpensive, does not require the use of endoscope.	Not distinguish between current and previous injury, as it is not used to confirm the effectiveness of treatmen
Stool antigen test	Available, fast, simple, inexpensive, does not require the use of periscope, accurate diagnosis. Used in the detection of infection before and after treatment. Suitable for the diagnosis of infection in childre.	May give false negative results due to the lack of bacterial numbers, the patient taking antibiotics and bismuth before examination, bloody bleeding. The sensitivity of the patient as well as laboratory personnel from dealing with feces.
Urea breath test	Simple does not require the use of periscope, safe, accurate, used to detect the elimination of injury.	An expensive and unavailable test may give false negative results due to bleeding or taking antibiotics.

with other antigens such as Campylobacter, especially in disease settlement sites (Patel *et al*, 2014). False positive results can be attributed to the survival of antibodies in the patient's blood for several months after treatment and recovery (Suerbaum and Michetti, 2002). The fecal antigen test showed the highest percentage of positive samples compared to the other test methods used in the current study to diagnose *H. pylori*. The results of our study showed that the use of fecal antigen test to diagnose *H. pylori* gave positive results of 93.6%, the highest percentage obtained compared to other screening methods used in this study. The results of our study were accordance to those results of Gold *et al* (2014), which recorded a 92% positive result of the fecal antigen test (Gold *et al*, 2014). However, the results of our study did not match (Dannoun and Al-Rawi, 2017), where they obtained positive results by 34% when investigating the level of antigen from the stool. Also, the current results disagreed with the study Hussein *et al* (2016), where the incidence of *H. pylori* in fecal antigen screening was 66.7%. The present results showed that the percentage of positive samples for the breath test was 90% of the patients and this is consistent with the results of the study of Richter *et al* (2019), which recorded 92.5% using UBT test to detect *H. pylori*. The current results disagreed with the results of Kawai *et al* (2019), which obtained a 72% rate using the breath test to detect *H. pylori*. Also, this results disagreed with the Perets *et al* (2019), which reached 46.9% of the positive samples using a breath test to detect *H. pylori*. The study by Furuta *et al* (2018) suggested that the sensitivity of the UBT test could be reduced by taking antibiotics such as Bismuth and proton pump inhibitor so patients should stop taking bismuth and proton pump inhibitors before testing. The UBT test may give a false negative result due to gastric bleeding, so the stomach should be cured by bleeding before a UBT examination (Velayos *et al*, 2012). In this study several methods were used to diagnose *H. pylori* bacteria, some of which require the use of endoscope to obtain the sample and others do not require the use of an endoscope. The positive and negative properties of each method can be illustrated in Table 2. This is consistent with several studies (Miftahussurur and Yamaoka, 2016; Talebi Bezmin Abadi, 2018; Sabbagh *et al*, 2019).

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