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Non invasive Methods as a rapid diagnosis of *H.pylori* infection

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Abstract:

Background: This study was carried out to determine the *Helicobacter pylori* (*H. pylori*) infection in patients who suffered from symptoms and signs of gastritis and gastric ulcer by using non-invasive techniques and to detect the highly sensitive, specific and accurate diagnostic tests.

Methods: A total of 38 serum and stool samples from patients presenting with symptoms of gastritis or peptic ulcer disease and 10 healthy volunteers were tested at Morjan Hospital, and private Laboratory from Al-Hilla city Babylon province from April 2016 to April 2017. Stool samples were examined for detection of *H. pylori* Ag (HpSA) using strip immune-chromatography method and presence of anti-*H. pylori* antibodies in serum by serological method and *VIDAS* instrument for titer of IgG.

Results: Among the 38 suspected patients, *H. pylori* were detected in serums of twenty one (21, 55.3%) by serological (immunochromatography Kit). From the stool samples of 38 patients twenty two samples were examined by serological kit device, the results were positive of HpSA in (25, 66%), also negative result appeared in 10 volunteers. *H. pylori* (IgG) antibodies also examined by VIDAS instrument the result were appeared positive in (23) patients with different titer.

Conclusion: We present and recommend a non invasive methods technique which has sensitivity and specificity in diagnosing *Helicopacter Pylori*. Using VIDAS instrument as rapid and accurate device is important in give titer of infection and may be used in follow up the patient after treatment.

Keywords: *H. pylori*, IgG , VIDAS, gastritis, peptic ulcer, HpSA,.

INTRODUCTION

Helicobacter pylori (*H.pylori*) is a micro-aerophilic Gram-negative bacterium with spiral shape. The helix shape of this is bacterium play a role in penetration the mucous layer of the stomach [1]. It is linked to the development of chronic gastritis, stomach ulcers, duodenal ulcers, and atrophy of stomach mucosa. Moreover, *H. pylori* cause a chronic inflammation and atrophy, so it is well recognized as a class I carcinogen and can further lead to malignant transformation. At least half the population in the world is infected by this bacterium especially the developing countries where the estimation rates are to be around 80% [2].

H. pylori is contagious infection, but the exact route of transmission is not known. Person-to-person transmission by either oral-oral or fecal-oral route is the most. *H. pylori* also may be transmitted by means of fecal matter through the ingestion of waste-tainted water. From many reportes, factors for *H. pylori* infection included poor hygiene, deficient sanitation, and in crowded communities [3]. However, the roles of many other factors associated have not been elucidated.

H. pylori recognized as related to Gastric adenocarcinoma which usually arises in the distal stomach area (antrum) in addition to the gastritis and peptic ulcer disease [4]. *H pylori* infection is involved in the pathogenesis of gastric adenocarcinoma and lymphoma in adulthood, in addition to their role in dyspepsia and extra-digestive diseases [5].

Diagnosis of *H pylori* can be made with both invasive and non invasive tests. Invasive tests, including histological, culture, and urese tests require endoscopy to obtain the biopsies from gastric mucosa. Although these tests are highly specific, sensitivity can be affected by the focal distribution of the infection within the stomach [6]. Now, non invasive tests have been developed to diagnosis *H pylori* and based on analysis of samples of breathing, blood, or stool [7]. All these tests are relatively inexpensive and rapid, but for validation, typically using histology or culture as the gold standard, must be performed initially [8].

The aim of this study:

- Detection of *H.pylori* infection in patients suffering from gastritis or gastric ulcer,
- Evaluate the non invasive method and compare between detection of H.pylori IgG Ab in serum and *H.pylori* Ag in stool samples.

SUBJECTS AND METHODS:

This study was carried out at Al-Mustaqbal University College samples were collected from Morjan Teaching Hospital and private laboratories from April 2016 to April 2017. Thirty eight (38) patients presenting with symptoms of gastritis or peptic ulcer disease, Ten (10) healthy volunteers who were never had any symptom of gastritis or peptic ulcer were included in the study as control group with approximately the same age of patients..

Samples collection: About 5-10 ml of blood was collected in disposable test tubes from patients and control group, serums were collecting by centrifugation of blood for ten minutes at 3500 rpm, serum were transferred to disposable test tubes. The serum or plasma sample was stored refrigerated at (2 –8 °C) for up to 48 hours. For a longer storage they were kept at -20 °C. The blood and serum then used for the several following criteria

H. Pylori Rapid Test Principle: This double antigen chromatographic lateral flow immunoassay very easy. The test strip includes a burgundy-colored conjugate pad containing colloidal gold coupled with *H. pylori* antigens and nitrocellulose membrane containing a test line (T line) and a control line (C line). T line is coated with H. pylori Ags, the C line is coated with goat anti-H. pylori antibody. The antigens used in this device are from H. pylori cell lysate [9]. When IgG antibodies specific to *H.pylori* are present in the specimen, the T line will become a burgundy-colored band. The C line should always appear as a burgundy-colored band regardless of the presence of antibodies to *H.pylori*. The C line serves as an internal qualitative control of the test [10].

Stool Test Principle: A fresh stool sample with approximately the size of a peanut was collected and stored at -20 °C for analysis [11].This assay is a double antibody chromatographic lateral flow immunoassay. The test strip include: a burgundy colored conjugated pad containing colloidal gold coupled with Pylori (out of membrane protein, OMP) polyclonal antibody, and nitrocellulose membrane containing a test line (T -line and a control line (c-line). The T line is coated with Pylori antibody .and the C-line is coated with goat anti-*H.pylori* antibody. The antigens

used in this device are from *H.pylori* cell lysate.When patient antigen to *H.pylori* are present in the specimen, The C line should always appear as a burgundy-colored band regardless of the presence of antigen to *H.pylori*.

VIDAS Instrument Rapid detection of *H.pylori* IgG in serum

VIDAS *H. pylori* IgG offers a non-invasive, reliable, cost-efficient method to determine the presence of anti-*H.pylori* IgG antibodies in human serum or plasma. It is an automated qualitative test for use on the instruments of the VIDAS® family by using the ELFA technique (Enzyme Linked Fluorescent Assay), it allows rapid detection of anti-*Helicobacter pylori* IgG antibodies in human serum or plasma Serological methods such as enzyme immunoassays are non-invasive, inexpensive, quick, and easy to perform. Compared to invasive methods**,** they do not rely on the accuracy of the sampling [12].

RESULTS AND DISCUSSION

Descriptive data on study subjects:

This study processed the serum and stool samples collected from peptic ulcer patients and gastritis. All patients in this study did not received antibiotic treatments previously. This study involved 38 peptic ulcer patients 22 males (58%) and 16 females (42%) gastric ulcer as shown in Table (.1); their mean ages was 42.1 ± years which range from 20 to 60 years , compared with eight samples taken from normal individuals that proved free from gastritis and ulcer and their mean ages was 48.75 .

Table (1). Distribution the patients according to the sex of patients.

Sex	No of patients	Percentage
Male	**22**	**58 %**
Female	**16**	**42 %**
Total	**N=38**	**100 %**

Distribution of H. pylori infection to the age and Sex of peptic ulcer patients

The occurrence of these disease **(peptic ulcer** and gastritis) were higher in males than in females, as shown in table (1), 22:38 (58%) were males and 16:38 (42%) were females as shown in the table(1). The results can be compared with other results obtained by [13], which found the prevalence of *H. pylori* was 68%; 69.6% in males and 66.7% in females. It is contrary to the science which proved that the rate of injury in women more than men suggesting that there was a significant difference between male and female [14]. The results showed high rate of incidence of age between 30-40 years old as show in fig (1).

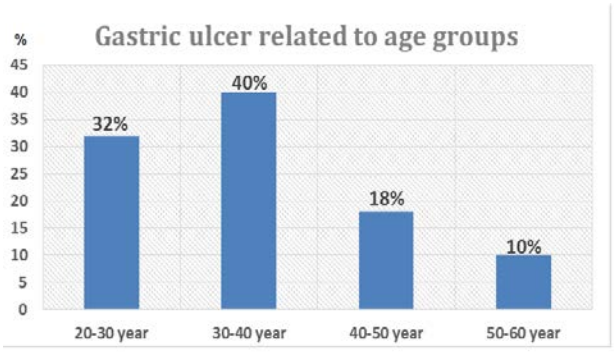

Figure (1) shows Distribution the patients according to the age.

Also, the result showed that the patients with blood group (A+) is most susceptible to the infection of the *H .pylorie* bacteria as shown in table (4.3), (A+14) (B+8) (AB+7) (O+9), the result proved by [15]. It is well known that blood group antigens are related to the development of peptic ulcer and gastric carcinoma. This study bought to determine the relationship between *H.pylori* and ABO blood group. All patients were randomly selected in each age group.

Prevalence of all blood groups showed that A (37.2%), B (21 %), AB (18 %) and O (24 %).As expected from previous studies, the result revealed that the patients in blood groups A and O were more prevalence to *H. pylori* infection than other patients in other blood groups and patients in the AB blood group were less prone to *H.pylori* infection between other blood groups.

Table(2) show the percentage of blood group

ABO Blood group	Number of patient	Percentage
A	14	37%
B	8	21%
AB	7	18%
O	9	24%
Total	38	100%

Laboratory investigations:

Serological methods:

Serological diagnostic methods were done using the test strip double antigen chromatographic lateral flow immunoassay and VIDAS by using the ELFA technique (Enzyme Linked Fluorescent Assay). The result showed that from 38 patients in this study 21, 55% of patients gave positive result using strip method where seventeen (17) showed negative from cases in this study.

By this study and other studies this method may be more safe than endoscope for rapid diagnosis patients with gastritis infected with this bacteria as show in the table 3.

Also another immunological method was used by VIDAS automated system which revealed (23) samples gave positive results (**TV ≥1.00**). TV is the test value =patient RFV (relative florecence value) /standerd RFV as shown in the table(4). In addition two (2) samples that gave negative result by strip method gave positive with VIDAS which add more accuracy for this device.

Table (3) Number and Percentages of positive and negative results for detection of *H.pylorie using* strip double antigen chromatographic lateral flow immunoassay

Serological Results	Number	Percentage (%)
positive	21	55%
negative	17	45%
Total samples	38	100%

Table 4. Number of positive and negative results for detection of H.pylorie by VIDAS system.

Serological Result	Positive results by VIDAS	Negative results by VIDAS	Total
Positive By Strip methods	21	0	21
Negative By Strip methods	2	15	17
Total	23	15	38

Detection of *H.pylorie* Antigen in the stool samples.

From thirty eight (38) samples collected from the same patients (26) samples gave positive results with percentage 68% by using strip immune-chromatography method and twelve (12) gave negative with percentage 32%. Only twenty (20) of samples gave positive results for both serological and stool non invasive methods as show in table 5.

Table 5. Number and Percentages of positive and negative result of the stool samples.

Stool Collection	Number	Percentage	Result of both *H.pylorie* Ag & *IgG*
Positive	26	68%	20
Negative	12	32%	18
Total	38	100%	38

For all three methods and tests performed in this study Sensitivity Specificity and accuracy in Percentages were measured by :
Sensitivity= (True Positive TP) / (True Positive TP + False Negative FN)
Specificity= (True Negative TN) / (True Negative TN + False Positive FP)
Accuracy= (TP + TN) (TP +FP + FN + TN)
And the results as in table 6 below.

Table 6. The results of Sensitivity, Specificity and accuracy in Percentages for the three methods.

The Method	Sensitivity %	Specificity %	Accuracy %
H.pylorie IgG by Rapid Kit	94.5 %	85.9 %	89.5 %
H.pylorie IgG VIDAS	88.4 %	93.7 %	91.3%
H.pylorie Ag in stool	89.6 %	85.7 %	87.1 %

DISCUSSION:

Depending of previously studies *H. pylori* is now considered to be the most prevalent infectious disease occurs in humans; about 50% of the human population is estimated to be infected. This type of bacteria can cause persistent gastritis and is directly linked to the development of peptic ulcer disease in addition to gastric adenocarcinoma and lymphoma of the stomach.

This study found that gastritis caused by *H. pylori* was significantly higher in studied age group between 30-40 years old, also the study found significant difference between both sex regarding the incidence of gastritis with *H. pylori* ,in addition blood group play important role for porn infection.

By this research the non invasive methods for detection of *H.pyloie* infection gave good results for rapid and safe easy diagnosis if it compare to other invasive methods like endoscope method. The present study was agreement with Andrews *et al* [8] and Robert *et al* [16] which they found nearly results at the same points of research. We evaluated the accuracy of immunochromatographic method to detect anti-*H. pylori* IgG in human serum and *H.pylorie* Ag in stool. In this study test strip for detection of *H.pylorie* Ag in stool & *H.pylorie IgG* Ab in serum and VIDAS® Kit are commercially available and inexpensive. In other study, it recommends these types of technique have sensitivity and specificity in diagnosing *Helicopacter Pylori* [11]. One of advantages of serological test is that the accuracy of serological tests is not affected by ulcer and gastric atrophy also the antibiotics use, which cause false negative results in other invasive or noninvasive tests. However, serological test not a reliable in result if we estimate the eradication therapy because the antibodies against Ag of bacteria can persist in the blood for periods of time even use successful treatment and eradication [17]. In *H.pylorie* infection there are difficult to distinguish between active infection and past exposure to *H. pylori*, so further confirmation by other tests is required before eradication therapy [18].

From the results of this study it revealed that specificity of using VIDAS for detection of *H.pyloie* infection gave high percentage compare to the others were the sensitivity high in rapid Kit and the differences in accuracy appeared low percentage between the three methods.

Also the study showed that stool Ag test can be used as good method for diagnosing *H. pylori* infection. This test with the accuracy about (87%) making it a suitable for the using in the clinical practice especially with signs of gastritis. However, this result applies only to this especial kit. Efficacy of stool Antigen tests to detect *H. pylori* infection depends mainly on the antigen selected by Kit for detection. A study by [5] demonstrates that among noninvasive and easily applicable tests, particularly in small children, *H pylori* fecal test is simple, suitable, and has high accuracy for the screening of *H pylori*.

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