ORIGINAL ARTICLE

Diagnosis of *Helicobacter pylori* infection in Assiut University Hospital and medical laboratories by using non-invasive tests

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ABSTRACT

Background: *Helicobacter pylori is a gram negative bacterium and considered as one of the causative factors of gastritis and peptic ulceration. The exact route of transmission is still unknown. H. pylori infection was highly prevalent worldwide.* ***Objectives****: to detect H. pylori infection rate by different tests including ELIZA tests for anti H. pylori antibodies (IgM, IgG, IgA) and H. pylori antigen, to isolate H. pylori by bacterial culture, to evaluate antimicrobial susceptibility of bacteria and to estimate bacterial infection rate according to clinical symptoms of patients.* ***Methodology****: This study was done on 130 stool samples and 130 blood samples collected from 130 patients attending Gastroenterology Department of Assiut University Hospital and some special medical laboratories, collected during the period from March 2019 to February 2020. Stool samples were processed to detect H. pylori antigen titers and to isolate H. pylori bacteria, blood samples were processed to detect anti H. pylori antibodies. Antimicrobial susceptibility of H. pylori was evaluated to ten antibiotics by disc diffusion method.* ***Results:*** *H. pylori infection rate was 86.1% by bacterial culture, 81.5% by anti-H. pylori IgG antibodies test , 48.5% by anti-H. pylori IgA antibodies test, 13.8% by anti-H. pylori IgM antibodies test, 76.9% by H. pylori antigen test. 39.2% of H. pylori infected patients had abdominal pain. Bacterial strains were resistant to several antibiotics.* ***Conclusion:*** *we concluded that anti-H. pylori IgG antibodies and H. pylori antigen tests were the preferable tests for diagnosis of H. pylori infection. According to statistical analysis, these results were more reliable and valuable. Abdominal pain was the most common symptom in H. Pylori infection. ciperofloxacin and amoxicillin were the most effective antibiotics for inhibition of bacteria.*

INTRODUCTION

Helicobacter pylori is a gram negative micro-aerophilic fastidious bacterium [1]. It was established as one of the causative factors of gastritis and peptic ulceration [2], so the discovery of *Helicobacter pylori* in 1982 was the beginning point of a revolution with regard to the concepts and management of gastro-duodenal diseases.[3]

This bacterium possess special characteristics that allow them to resist the very acidic environment of the stomach. For example, flagellum that promotes its mobility to the mucus layer at the surface of the gastric mucosa, causing the bacterial colonization, inflammation, and immune evasion, it produces urease, an enzyme that contributes bacterial colonization and this enzyme is used as a biomarker of *H. pylori* infection as part of the rapid urease test. [4]

The exact route of transmission is still unknown, despite multiple attempts to reveal the mechanism. Because crowding settings is a leading risk factor for infection and *H. pylori* infection occurs mainly within families and person-to-person spread is the most likely mode of transmission. Fecal-oral, oral-oral, and gastro-oral transmission routes are possible, particularly since *H. pylori* can be isolated from feces, saliva, and vomitus respectively.[5]

Several studies have cleared that the worldwide prevalence of *H. pylori* infection is high, and some of them have estimated that 50% or more of the world's population is infected. [6]

The obvious symptoms of *H. pylori* infection are relatively nonspecific, such as epigastric pain, postprandial fullness, bloating, nausea, and vomiting, along with signs of acid hyper-secretion and delayed gastric emptying [7,8].

There are several factors such as age, gender, ethnicity, and a variety of socioeconomic status are associated with the prevalence *of H. pylori* infection and overcrowded settings, low socioeconomic status is one of the essential factors that renders children at risk for acquiring the infection.[5]

Several treatment regimens are present, the triple therapy is the standard treatment regimes for *H. pylori* infections comprising two antibiotics and a proton pump inhibitor (metronidazole, clarithromycin, levofloxacin or amoxicillin combined with omeprazole or pantoprazole) Quadruple therapy is an alternative treatment including bismuth salts in combination with the existing antibiotics .[9]

METHODOLOGY

One hundred and thirty blood and stool specimens were obtained from 130 patients (ages from 15 to 65 years; mean ± 12.6 years) referred to Assuit university hospital (the Gastroenterology Department) and some medical laboratories with some gastrointestinal symptoms such as: abdominal pain, nausea and heartburn.

The study was approved by the ethical committee of the faculty of Science, Sohag University and a confirmed consent was obtained to take blood and stool samples from patients.

Serum anti-*H. pylori* (IgG, IgA and IgM) antibodies and *H. pylori* stool antigen(Hp SA) were estimated with a commercial enzyme-linked immunosorbent assay (ELISA). It's based on an enzyme immuno-assay technique with partially purified *H. pylori* bacteria antigen absorbed on a microplate and a detection antibody labeled with horse radish peroxidase (HRP). The procedures were performed according to manufacturer's instructions. The assay results were categorized as follows: negative: 0.9, Border line positive: 0.9-1.1, positive: >1.1 U/ml for IgG antibodies and Hp SA, negative: < 20.0, Border line positive: = 20.0, positive: > 20.0 U/ml for IgA and IgM.

Fifty mg of feces were homogenized in 1 ml sterile saline solution. The homogenized specimen was streaked on Columbia blood agar base plates augmented with campylobacter supplement- III (Skirrow) and incubated at 37°C under micro-aerobic conditions (5% oxygen (O_2), 10% carbon dioxide (CO_2), and 85% nitrogen (N_2) with 100% humidity) up to 7 days. The bacteria were identified by Gram staining, colony morphology, oxidase, catalase and urease reactions.

Suspensions of *H. pylori* cultures were adjusted to a McFarland standard as the inocula on Mueller Hinton agar plates were augmented with 5 % sheep blood then the disks of different antibiotics such as: Tetracycline (30µg), clarithromycin (15µg), ciprofloxacin (5µg), Levofloxacin (5µg), Amoxicillin (10µg), Rifampin (5µg), Gentamicin (10µg), Erythromycin (10µg), Furazolidone (30µg) and Metronidazole (5µg) according to National Committee for Clinical Laboratory Standards (NCCLS)[10]. They were placed on the plates and were incubated for three days then inhibition zones were measured in millimeters. The zones of inhibition were interpreted according to the standard of the Clinical and Laboratory Standard Institute (CLSI) [10,11].

RESULTS

Table 1: Detection of *H. pylori* antibodies and antigens in patients underlying the study:

Tests	Positive results		Negative results	
	No. of samples	%	No. of samples	%
IgG	106	81.5	24	18.5
IgA	63	48.5	67	51.5
IgM	18	13.8	112	86.1
H. pylori Stool Antigen	100	76.9	30	23.1
Culture(stool)	112	86.1	18	13.8
Total no. of samples	130			

From hundred and thirty blood samples, hundred and six samples were positive for IgG (81.5%) and hundred samples were positive *H. pylori* Ag (76.9%). Hundred and twelve samples were positive *H. pylori* by bacterial culture (86.1%). Tab (1)

Table 2: Descriptive Statistics of the four tests (IgG, IgA, IgM, H. pylori Ag) concerning gendre and site:

	IgG		IgA		IgM		H. pylori Ag	
		Std. error		Std. error		Std. error		Std. error
Mean	3.035	0.1684	20.0400	0.63331	14.5677	0.59441	2.8662	0.15654
Maximam	7.9		46.6		41.8		7.1	
Minimum	0.1		4.2		4.1		0.1	
Std. deviation	1.9198		7.22090		6.77727		1.78480	
***P*- value**	0.000		0.000		0.000		0.000	

From the descriptive statistics, the values of mean for IgG, IgA, IgM and *H. pylori* Ag were variables (3.035, 20.0400, 14.5677, 2.8662) that indicated the results were valuable. The variation for IgG values from its mean (standard deviation) and standard error are 1.9198 and 0.1684 in Assiut. The variation for IgA values from its mean and standard error are 7.22090 and 0.63331 in Assiut. The variation for IgM values from its mean and standard error are 6.77727 and 0.59441 in Assiut. The variation for H. pylori Ag values from its mean and standard error are 1.78480 and 0.15654 in Assiut. This means that IgG and H. pylori Ag values in Assiut are more variable and reliable to detect the infection than others because standard deviation of them are low and approximate and the values of all variables for Assiut university hospital and some medical laboratories (IgG, IgA, IgM and *H. pylori* antigen) are accurate because of detection of low standard error. Table (2)

Table 3: Sensitivity, specificity and accuracy of *H. pylori* antibodies and antigen detecting tests depending on bacterial culture as gold standard:

Tests / No. of samples	Abs			Ag	Bacterial culture
	IgG	IgA	IgM		
True positive	102	57	14	94	112
True negative	14	12	14	12	18
False positive	4	6	4	6	----
False negative	10	55	98	18	----
False positive%	22.2%	33.3%	22.2%	88.9%	----
False negative%	8.9 %	49.1%	87.5%	16.1%	----
Specificity (True negative %)	82.3%	66.7%	77.8%	66.7%	100
Sensitivity (True positive %)	91.1%	50.9%	12.5%	88.7%	100
Accuracy %	89.2%	53.1%	21.5%	81.5%	100

In our study, the sensitivity of serological tests was 91.1%, 50.9% for IgG and IgA respectively, specificity was 82.3%, 66.7% while sensitivity of *H. pylori* Ag test was 88.7%, and specificity was 66.7%. Table (3)

Table 4: prevalence of *H. pylori* infection according to anti *H. pylori* IgM, IgG, IgA antibodies and *H. pylori* antigen tests and bacterial culture from blood and stool samples of patients of Assiut university hospital and some medical laboratories

	Assiut university														
Tests / Patients no.	Abs (quantitative)									Ag (quantitative)			Bacterial culture		
	IgG			IgA			IgM								
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
positive patients	61	45	106	33	30	63	11	7	18	52	48	100	65	47	112
%	46.9%	34.6%	81.5%	25.4%	23.1%	48.5%	8.5%	5.4%	13.8%	40%	36.9%	76.9%	50%	36.1%	86.1%
Negative patients	7	17	24	35	32	67	57	55	112	16	14	30	3	15	18
%	5.4%	13.1%	18.5%	26.9%	24.6%	51.5%	43.8%	42.3%	86.1%	12.3 %	10.8%	23.1%	13.9%	11.5%	2.3%
Total patients	130			130			130			130			130		
Positivity %	81.5%			48.5%			13.8%			76.9%			86.1%		
Negativity %	18.5%			51.5%			86.2%			23.1%			27.7%		

Prevalence of *H. pylori* infection was larger in males than females through all diagnostic tests 46.9% for anti *H. pylori* IgG test, 25.4% for anti *H. pylori* IgA test, 8.5% for anti *H. pylori* IgM test, 40.0% for *H. pylori* Antigen test and 50.0% for bacterial culture. Table (4)

Table 5: Occurrence of *H. pylori* infection between patients according to their symptoms:

Symptoms	No. of male	Occ %	No. of female	Occurrence %	No. of patients	Occurrence %
Abdominal pain	33	25.4	18	13.8	51	39.2
Nausea	29	22.3	19	14.6	48	36.9
Heart burn	29	22.3	12	9.2	41	31.5
Abdominal pain + Nausea	10	7.7	5	3.8	15	11.5
Abdominal pain + Heart burn	17	13.1	5	3.8	22	16.9
Nausea + Heart burn	8	6.1	5	3.8	13	10.0
Abdominal pain + Nausea + Heart burn	4	3.1	2	1.5	6	4.6
Total no. of Studied patients	130					

The symptoms of *H. pylori* infection includes abdominal pain, heart burn and nausea, 39.2% patients with *H. pylori* infection had abdominal pain, 36.5% had nausea and 31.5% had heartburn Table (5).

Table 6: The susceptibility pattern of *H. pylori* strains isolated from stool specimens to different antibiotics:

antibiotics	Sensitive strains number	Resistant strains number	Sensitivity rate %	Resistance rate %
AMX	89	9	90.8	9.2
CRP	86	12	87.8	12.2
ER	6	92	6.1	93.8
FURA	59	39	60.2	39.8
GN	13	85	13.3	86.7
LVX	41	57	41.8	58.2
MNZ	20	78	20.4	79.6
RIF	8	90	8.2	91.8
TCN	34	64	34.7	65.3
CAM	43	55	43.9	56.1

The resistance to metronidazole 79.6%, rifampicin 91.8% and gentamycin 86.7% were the highest while resistance to ciprofloxacin 12.2% and amoxicillin 9.2% was the lowest. The resistance of *H. pylori* strains to macrolides was cleared in this study (erythromycin 93.8% and clarithromycin 56.1%). Resistance to clarithromycin was less than erythromycin. Table (6)

DISCUSSION

Helicobacter pylori is classified as a Group I carcinogen[12], *H. pylori* infection is a definite and controllable factor in the development stages of gastric cancer, it is essential to investigate the prevalence rate in a large-scale population [13]

Laboratory tests of *H. pylori* infection are very important for the diagnosis process of various gastric diseases caused by *H. pylori*. A number of different diagnostic methods, both invasive and non- invasive are available [14]. The antibody detection is cheap and easy to perform [15], but may give false positive results. The stool antigen (Ag) test has recently become more acceptable as it is non-invasive, convenient for patients and can be performed easily even in small laboratories.[14]

H. pylori antigens tests could distinguish actively infected from treated patients that confirmed by results of many other studies [16] and the serology assay was a confirmed method to expect the severity of gastro-intestinal diseases or the rate of *H. pylori* colonization [17] but serology assay cannot differentiate between active and asymptomatic colonization [18] while the use of serological tests based on the determination of serum levels of anti-*H. pylori* IgG and IgA antibodies to clinically diagnosed *H. pylori* infection has not yet been fully clarified according to some researches. [19]. The activity of infection was predicted from the association of high activity of *H. pylori* Ag titers and the increasing titers of IgA, consequently the detection of specific antibodies to *H. pylori* in serum is substantial in the diagnosis of gastro-intestinal infections caused by *H. pylori*. [20]

This study confirmed that these diagnostic tests to *H. pylori* infection gave accurate, reliable and non-random

results according to descriptive statistics. IgG and *H. pylori* Ag values in Assiut are more variable and reliable to detect the infection than others because standard deviation of them is low and the values of all variables (IgG, IgA, IgM and *H. pylori* antigen) are accurate because of detection of low standard error. Anti *H. pylori* IgG antibody and *H. pylori* Ag tests are commonly more prevalent and major indicators to *H. pylori* infection.

Some researchers concluded that ELISA test for the detection of anti-*H. pylori* IgA and IgG antibodies was sensitive and specific and should preferentially be utilized in population-based studies. The sensitivity and specificity of ELISA test ranged from 75% to 95% some studies [21]whlie the sensitivity and specificity of ELISA test was 91.1% 82.3% for anti *H. pylori* IgG antibody test and 88.7% and 66.7% for *H. pylori* antigen test in our study. This difference in sensitivity and specificity may be due to the different methods utilized as the gold standard .[19]

In addition to other factors that potentially decrease the sensitivity and specificity of results and affect the performance of a test used to screen patients including the precision of the test used to detect presence or absence of the infection, the methodology used to screen the infection [17], recent ingestion of antibiotics, bismuth compounds, and proton pump inhibitors [22], these agents altered gastric mucosal inflammation, bacterial distribution and make the detection of *H. pylori* more difficult so the chances for false negative *H. pylori* infection status were elevated [23,24,25].

Egypt is considered one of the highest prevalence areas for *H. pylori* infection and higher resistance to antibiotics due to the abuse of antibiotics. The prevalence in Egypt was 90 % in adults while 60-90% in Middle East as reported by the World Gastroenterology Organization (WGO). [26,27] Prevalence of *H. pylori* infection between patients ranged from 68.4% to 86.1% using different types of diagnostic tests. Our study was in consistent with a few previous studies in Bhutan (South Asia) which reported high prevalence of *H. pylori* infection ranged from 66.2% to 86%. [28]

H. pylori infection was more common in males than females (50% males and 36.1% females using the bacterial culture test). This predominance resulted from relative immunodeficiency under effect of hormones in males [29,30]. In our study, some symptoms were common in patients with *H. pylori* including abdominal pain, nausea and heartburn, however the role of *H. pylori* infection in the etiology of abdominal symptoms remains ambiguous [31]. From the results we found that the prevalence of *H. pylori* infections was higher in separate symptoms than in the gathered symptoms, these results matched to Bu¨yu¨kbaba-Borala *et al* [32] study that appeared *H. pylori* infections in patients with separate symptom (with stomach pain 33.7%) were higher than in patients with grouped symptoms (with stomach pain + heart burn 15.3% and stomach pain + nausea + vomiting 21.5%).The severity of clinical symptoms of H. pylori infection is associated to variable virulence factors such as Vac A, Cag A, Ure A, Dup A.[27]

Resistance (primary and acquired) of *H. pylori* strains may play a considerable role in treatment failure [33] and is the main factor affecting the efficacy of the current regimens [34].

H. pylori strains were resistant to metronidazole, rifampicin, erythromycin, clarithromycin and gentamycin while they were inhibited by ciprofloxacin and amoxicillin. Resistance to clarithromycin was less than erythromycin. This was due to clarithromycin penetrates the outer membrane better than erythromycin and achieves a faster rate of intracellular accumulation [35]. Moreover, the activity of clarithromycin is less influenced by acidity than that of erythromycin [36,37].

The bacterial resistance was the result of selection due to antibiotic pressure. [34] and the dominant use of antibiotics for treatment of *H. pylori* infection which gradually increases the phenomenon of antibiotic resistance [38].Therapies in patients infected with resistant strains are likely to fail and subsequent treatment will be more difficult [39,40].

CONCLUSION

Continuous developments in the methods for diagnosis of *H. pylori* infection will greatly contribute to further improvement of the health management of *H. pylori* associated diseases. amoxicillin and ciprofloxacin were preferable antibiotic in treatment until now.

This study was approved by the ethical committee of Faculty of Science.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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