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RESEARCH ARTICLE

HELICOBACTER PYLORI INFECTION IN EGYPTIAN PATIENTS WITH DYSPEPSIA: DIAGNOSTIC, DEMOGRAPHIC, ENDOSCOPIC AND CLINICAL CHARACTERISTICS.

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Abstract

Background and Study Aims: Helicobacter pylori (H. pylori) is considered as a public health problem, especially in developing countries. Understanding the epidemiological aspects of *H. pylori* infection is important and helpful in clarifying the consequences and complications of infection. The aim of this study was to assess the prevalence of *H. pylori* infection in dyspeptic patients and to study the demographic, endoscopic and clinical characteristics of *H. pylori* infected patients.

Patients and Methods: A total of 113 adult patients with dyspepsia were enrolled in this study. They underwent upper gastrointestinal endoscopy for obtaining four ~~antral~~ biopsies, patients were considered to be infected with *H. pylori* when they had positive results of rapid urease and/or *H.pylori* stool antigen tests and confirmed by detection of *H. pylori* 16S rRNA gene in the extracted DNA from gastric biopsy specimens by Polymerase Chain Reaction (PCR) assay.

Results: Sixty (53.1%) dyspeptic patients (17-76 years old) were confirmed to be infected with *H. pylori*. Age, sex, smoking history and taking spicy food had no significant correlation to the acquisition of *H. pylori* infection. Considering the PCR assay on gastric biopsy specimens as the gold standard, excellent agreement was found with both rapid urease and *H. pylori* stool antigen tests. Rapid urease test (91.7%) was more sensitive than *H. pylori* stool antigen test (83.3%), while both tests have specificity of 100%. Upon endoscopy; gastritis was revealed in 27(45%) and 10 (16.7%) had peptic ulcer disease (PUD).

Conclusions: H. pylori infection rate in Egyptian patients with dyspepsia was high and gastritis was the most revealed finding upon endoscopy. No risk factors were associated with *H. pylori* infection among the studied adult patients. Combined rapid urease and stool antigen tests can be relied upon for detecting *H. pylori* infection.

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Introduction:-

H. pylori, a microaerophilic, Gram-negative and spiral bacterium, is colonizing approximately 50% of the world's population and over 80% of individuals infected with *H. pylori* are asymptomatic [1]. It plays a significant role in the etiology and pathogenesis of peptic ulcer disease and increases the risk of developing gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma [2]. *H. pylori* is frequently associated with the symptom of dyspepsia which is considered as one of the most common upper gastrointestinal complaints [3].

The prevalence of *H. pylori* infection differs significantly among countries, with higher prevalence (20-90%) in developing areas compared to developed countries (10-60%) [4,5,6]. Several studies from Egypt reported that the prevalence of *H. pylori* infection ranged from 60-90% [7,8,9]. Adult factors associated with prevalence of *H. pylori* are poorly characterized and, in particular, it is unclear whether socioeconomic conditions, lifestyle and household overcrowding in later life are independent predictors of infection [10, 11]. The geographic variations, in addition to genetic heterogeneity of the host further contribute to the diversity of host responses to particular *H. pylori* strains and genotypes [11].

Several diagnostic tests for detection of *H. pylori* have been widely used in clinical practice. However, each of these tests has certain disadvantages [12].These diagnostic methods may be classified as invasive, which require endoscopy to obtain biopsies of gastric tissues, and non-invasive**.** The invasive methods include histological examination, culture, urease test and molecular methods, while the non-invasive methods include urea breath testing, serology and stool antigen testing. There is no single method that can meet, on its own, the criteria for acceptable sensitivity and specificity in identification of the bacterium. In the last few years, more interest has been paid for the non-invasive methods [13].

The aim of this study was to assess the prevalence of *H. pylori* infection in dyspeptic patients and to study the demographic, clinical and endoscopic characteristics of *H. pylori* infected patients. We tried to evaluate the role of combined testing assays for *H. pylori* diagnosis.

Patients and Methods:-

A total number of 113 adult patients with various symptoms such as dyspepsia (upper abdominal discomfort or pain) vomiting and/or heartburn and undergoing upper gastrointestinal endoscopy at the Endoscopy Unit, Theodor Bilharz Research Institute (TBRI) Hospital from March, 2013 to December, 2015 were enrolled in this study. Patients who had received non-steroidal anti-inflammatory drugs, as well as antibiotics, H2 receptors antagonists or proton pump inhibitors (PPI) four weeks prior to the study were excluded. All dyspeptic patients who underwent upper endoscopy and fulfilled the inclusion criteria were subjected to complete history (age, sex, residence, complain and habits). ~~Antral~~ Biopsy specimens were obtained from each patient for diagnosis of *H. pylori* infection. A patient was considered to be infected with *H. pylori* when he had positive results of rapid urease and /or *H. pylori* stool antigen tests and confirmed by detection of 16S rRNA in gastric biopsy specimens by PCR assay. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6^{th} revision, 2008) as reflected in a prior~~i~~ approval by TBRI institutional review board (FWA00010609) and all patients provided a written informed consent.

Sample Collection

Gastric biopsies: Upper gastrointestinal endoscopy was performed using an Olympus X Q40 endoscope. Upper endoscopic examination of the esophagus, stomach and duodenum, abnormalities (gastritis, ulceration, erosion and others) were recorded. From each patient, four biopsies were obtained from the antrum and corpus in 2 tubes. One tube was tested for rapid urease test and the other tube was stored in sterile physiological saline in sterile Eppendorf tubes and kept at -70°C until processed as panel for DNA extraction were used directly for PCR assay.

Stool specimens: One stool specimen was taken from each patient to be tested for detecting *H. pylori* antigen. Stool specimens were collected in sterile plastic cups and kept at -70°C until processed.

Rapid urease liquid test:

H. pylori urease production in gastric biopsy was detected using rapid urease liquid test kit (Bussero, Milan, Italy).

Detection of H. pylori antigen in stool specimens:

H.pylori antigen in stool specimens was detected using Immunospec Corporation Kit (Netherlands). The test utilizes purified anti-*H. pylori* capture antibody to detect *H. pylori* antigen in diluted stool specimens [14].

A standard curve was constructed for calculation of *H. pylori* stool antigen results by plotting the optical density on the y-axis against the concentration of the calibrator ng/ml values on the x-axis. Negative results: < 15 ng/ml while positive results: > 20 ng/ml.

DNA extraction and detection of H. pylori 16S rRNA gene by conventional PCR assay:
Genomic DNA was extracted from gastric biopsy specimens using QIAamp DNA min kit (Qiagen, USA) according to manufacturer guidelines. PCR assay was performed in a volume of 50ul with approximately 5 µg of extracted DNA, 200 µM (each) dNTPs, 25 pmol for each primer (Table 1), 1.5 µM Magnesium Chloride and 1unit of *Taq* polymerase (Gotaq Flexi DNA, M8305, Promega, Inc, USA) in PCR buffer. The reaction was done in PTC-100^{TM} thermal cycler (MJ Research, USA), programmed as follows: denaturing at 95°C for 5 min, followed by 37 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min, and final extension at 72°C for 5 minutes. PCR product was separated on 2% agarose gel with ethidium bromide, and 50bp ladder used as DNA molecular weight standard. In PCR assay, a negative control (lacking DNA) was included. PCR products were analyzed under UV light. Size of expected amplicon was 110bp [15].

Statistical analysis:
Data were described in terms of frequencies (number of cases) and relative frequencies (percentages). P-value < 0.05 was considered as statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, U.S.A.).

Table 1:- Primer used for PCR analysis of *H. pylori 16S rRNA* gene

Target gene	**Primer sequence (**5'-3'**)**	**Amplicon bp**	**Reference**
16S Rrna	5'CTG GAG AGA CTA AGC CCT CC-3' 5'ATT ACT GAC GCT GAT TGT GC-3'	110	Chisholm et al[15]

Results:-

Diagnostic performance of different assays used for diagnosis of H. pylori infection:
Regarding results of different assays used for diagnosis of *H. pylori* infection among 113 dyspeptic patients included in the study; for conventional PCR assay, 60 (53.1%) patients showed positive *H. pylori* DNA in gastric biopsy specimens, 55(48.6%) gastric biopsy specimens had positive rapid urease test and 50 (44.2%) patients had positive *H. pylori* stool antigen. The diagnostic performance analysis of different assays on gastric biopsies and stool specimens, considering PCR assay on gastric biopsy specimens as the gold standard, was; 60 (53.1%) patients were diagnosed to be infected with *H. pylori* by positive results of rapid urease and /or *H. pylori* stool antigen tests and confirmation by detection of 16S rRNA in gastric biopsy specimens. The sensitivity rate of rapid urease test (91.7%) was higher than that of *H. pylori* stool antigen test (83.3%) and for specificity rate of both tests was 100%. Excellent agreement was found with rapid urease test and stool antigen test, with Kappa coefficient of 0.912 and 0.824; respectively (Figure 1 and Table 2)

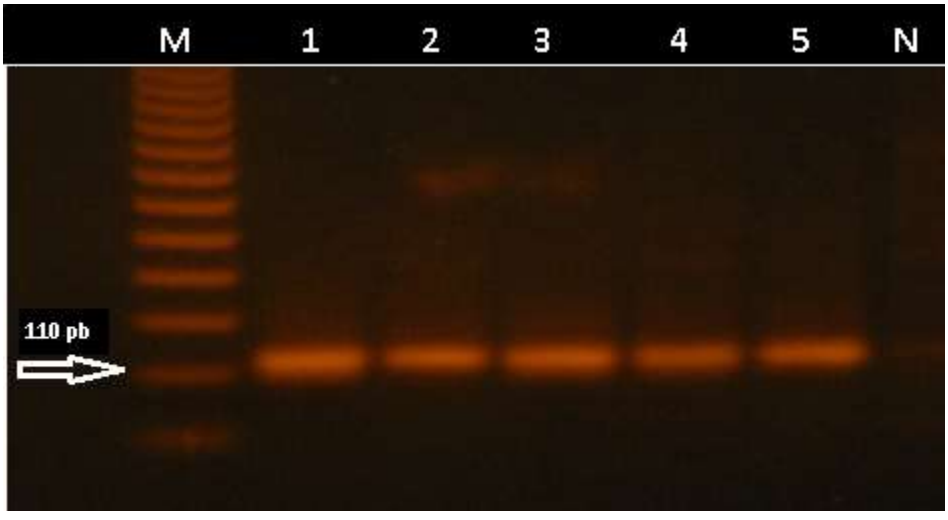

Figure (1):- Agarose gel electrophoresis of PCR products of *H. pylori 16S rRNA* positive gene (110 bp) from gastric biopsy specimens on agarose gel. Lane M: molecular weight marker (ladder 50 bp). Lanes (1-5): Positive cases of *H.pylori* possessing *16S rRNA* gene. Lane N: negative control

Table 2:- Diagnostic performance of biopsy-based rapid urease and stool antigen tests among the studied 60 *H. pylori* infected patients

Test **(n=113)**	**PCR assay on gastric biopsy**		**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Efficacy**	**Kappa value**
	Positive (n= 60)	**Negative** (n= 53)						
Rapid urease test								
Positive (n= 55)	55 (91.7%)	0 (0.0%)	55/60 (91.7%)	53/53 (100%)	55/55 (100%)	53/58 (91.4%)	108/113 (95.6%)	0.912**
Negative (n= 58)	5 (8.3%)	53 (100%)						
Stool antigen test								
Positive (n= 50)	50 (83.3%)	0 (0.0%)	50/60 (83.3%)	53/53 (100%)	50/50 (100%)	53/63 (84.1%)	103/113 (91.2%)	0.824**
Negative (n= 63)	10 (16.7%)	53 (100%)						

Data are expressed as number (%), PPV= Positive predictive value, NPV= Negative predictive value ~~**p< 0.01= Highly significant~~

Demographic features of the studied patients:

Among the studied 60 *H. pylori* infected patients, 40 (66.7%) patients were males and 20 (33.3%) were females (P value =0.26).The patients' ages ranged between 17 and 76 years (49.93 ± 14.28 years), and 85% (51/60) patients were from urban region while 15% (9/60) were from rural region (P value=0.97). Half of the *H. pylori* infected patients (31/60, 51.7%) were above 51years, 46.7% (28/60) were between 21 and 50 years, while only one patient (1.7%) was below 20 years (P value > 0.5). *H. pylori* infection increases with age, and was possibly found more in population above 51 years.

Risk factors associated with H. pylori infected patients:

The risk factors associated with *H. pylori* infection include smoking and eating spicy food (13/60; 21.7%; each) and both factors were detected in 10 % (6/60) of patients (P value > 0.5).

Table 3:-Risk factors associated with *H.pylori* infected patients

	***H. pylori* Infected Patients (n=60)**	
	No.	**%**
Risk factors		
Smoking history	13	21.7
Eating spicy food	13	21.7
Smoking history and eating spicy food	6	10

Data are expressed as number (%)

Clinical and Endoscopic Findings of the Studied Patients:

Most of the studied 60 *H. pylori* infected patients presented mainly with dyspepsia 34(56.7%) followed by vomiting 16(26.7%) then heartburn 10(16.7%). Upon upper gastrointestinal endoscopy of the gastroduodenal mucosa *H. pylori* infected patients revealed gastritis (hyperemic mucosa) in 45%, whereas 16.7% of such patients have peptic ulcer disease. Other endoscopic findings as antral erosion, gastric prolapse, GERD, esophageal varices, esophagitis and hiatus hernia were detected in 31.7% patients and 6.7% of patients had apparently normal gastric mucosa (Table 4).

Table 4:- Clinical and Endoscopic findings among *H. pylori* infected patients

	***H. pylori* Infected Patients (n=60)**	
	No.	**%**
Clinical findings		
Dyspepsia	34	56.7
Vomiting	16	26.7
Heart burn	10	16.6
Endoscopic Findings		
Gastritis	27	45.0
Peptic Ulcer	10	16.7
Apparently Normal Gastric Mucosa	4	6.7
Others (antral erosion, gastric prolapse, GERD, oesophageal varices, oesophagitis and hiatus hernia)	19	31.7

Data are expressed as number (%)

Discussion:-

H. pylori infection prevalence rates vary from country to country and differ greatly among population groups within the same country. It can be due to diverse contributing factors including socioeconomic status, geographical or living conditions and location of each population [4, 5, 16]. In the current study, 53.1% of the studied patients were *H. pylori* infected positive by rapid urease test and/or stool antigen test and confirmed by detection of *16S rRNA* gene by PCR assay in panel of gastric biopsy specimens. Comparable results *H.pylori* infection were recorded by Goh [17] from Malaysia (49%), by Alazmi et al [18] Kuwait (49.7%) and by Hasosah et al [19] from Saudi Arabia (49.8%). Previous Egyptian studies recorded higher results; Diab et al [7] detected *H. pylori 16SrRNA* gene in a rate of 64.3% in gastric biopsy specimens by PCR assay; Ali and Borei [20] and Abu-Zekry et al [21] recorded *H .pylori* infection of 62% and 70% using C13-urea breath test and *H. pylori* antigen detection in stool respectively. A recent Egyptian study by El-Khlousy et al [22] reported that *H. pylori* infection was detected in 62.2% by histopathology while 75.7% were positive for *H. pylori* 16S rRNA gene by PCR. Egypt had the highest prevalence of *H. pylori* in the healthy asymptomatic population both in adults and the pediatric age group**.** Low socioeconomic status, low body weight and height, living in rural areas and lower educational status were risk factors for the acquisition of *H. pylori* in Egyptian studies [23]. In contrast, studies which reflect good hygienic conditions and less crowded environments reported a much lower prevalence from several European countries [16, 24].

In the current study, 85% of *H. pylori* infected patients were from urban region where these patients are living in a low-income urban region with low educational level. Similarly, previous studies reported higher prevalence rates of *H. pylori* infection in low-income urban areas [10]. Jaka et al [24] also reported significantly higher prevalence rates of *H. pylori* infections in rural areas compared to urban areas.

In the present study, although the prevalence of *H. pylori* infection was higher in males, however, no statistically sex difference was found as it has also been found in other studies conducted in developing countries, there were no gender differences in the risk of acquisition of infection [18]. On the contrary Niknam, et al [25], reported predominant *H. pylori* positivity in females.

Age was shown to have an effect on the prevalence of *H. pylori* infection with lower rates in subjects younger than 20 years old [26]. Ford and Axon [27] reported that *H. pylori* infection prevalence increases with age between 7% and 87%. Among adults, the *H. pylori* infection rate increases with age [24].The current study the prevalence of *H. pylori* infection increases with age; however the differences were not significant. Hu et al [28] reported that the highest prevalence of *H. pylori* was in 40-49 years age group, they explained that these subjects always tend to dine out which increases the chance of exposure to *H. pylori* infection.

Smoking is a social stigma that is fast growing into an epidemic. It has been elucidated in molecular studies that smoking causes the expression of *cagA* gene in the gastric milieu. This gene could play a role in the transformation of an ulcerated gastric lining into a malignant tumor [29]. In the current study, smoking was associated with *H. pylori* infection in 21.7%. Such result was contradictory to Bakka and Salih [26] study and was consistent with previous study by Hu et al [28] who reported that the use of tobacco had been shown to impair the immune system, therefore contributing to an increased chance of *H.pylori* infection.

H. pylori infection could also be related to eating habits [30]. In the current study, eating spicy food, as a risk factor, is associated for *H. pylori* infection. These results were in agreement with Hu et al [29] who reported that spicy food and pickle food are risk factors for *H. pylori* infection. Spicy food and pickle food may decrease the ability of gastric mucosa to prevent *H. pylori* infection and a high intra-gastric salt content may destroy the mucosal barrier, leading to inflammation and damage such as diffuse erosion and degeneration in the stomach.

Dyspepsia is a common gastrointestinal disorder and could present as dysmotility or ulcerative like dyspepsia. It is considered as the most common indication for gastric upper endoscopy [31]. The current study showed that 56.7 % of the *H.pylori* infected patients were presented with dyspepsia. Such results were lower than those found by Vilaichone et al [32] from Thailand. In the Western World, the prevalence of dyspepsia was ranged between 25 to 50% [25]. Savarino et al [33] documented that up to 70% of reflux patients have typical reflux symptoms (i.e. heartburn and/or regurgitation) in the absence of endoscopic visible oesophageal mucosal injuries.

More than 50% of the world's population is infected with *H. pylori* [27], however, 10% only will develop peptic ulcer disease and1%–2% will develop gastric malignancy [34]. The upper gastrointestinal endoscopy of the studied *H. pylori* infected patients revealed that, the most common findings were gastritis (45%) followed by peptic ulcer (16.7%). Our results were in agreement with Ngoyi et al [35] and lower than Rasheed et al [36]. Feliciano et al [37] reported that during upper endoscopy, patient's mucosa was distributed into non-peptic ulcer dyspepsia in 64.7% and peptic ulcer in 35.3%of cases. Other endoscopic findings (31.7%) were observed among the studied *H. pylori* infected patients. Moschos et al [38] showed that *H. pylori* eradication may positively influence GERD symptoms. Ngoyi et al [35] had detected hiatal hernia in 1.6% of the examined cases. Another study from Egypt by Safwat et al [39] showed that there is significant association between *H. pylori* infection and the occurrence and the severity of portal hypertensive gastropathy (which may be associated with esophageal varices) in patients with HCV-related liver cirrhosis, thus, eradication of *H.pylori* may be beneficial to improve portal hypertensive gastropathy.

Of the several available diagnostic tests for detection of *H. pylori* infection, the choice depends on the sensitivity, specificity, reproducibility, availability cost, and rapidity of the results**.** There is a need for a reference method to be used as "gold standard" to detect patients truly infected. Unfortunately, none of the currently used methods is able to further this criterion [40]. Nowadays, studies show that the PCR assay may be slightly superior to other diagnostic methods for detection of *H. pylori* from different clinical samples and to verify the bacterium eradication after treatment. The need for a limited amount of bacteria enables PCR to recognize infection when other tests are negative due to low bacterial density [41].

In the current study, 53.1% of the studied patients were *H. pylori* positive by rapid urease test and/or stool antigen test and confirmed by detection of *16S rRNA* gene by PCR assay in panel of gastric biopsy specimens. We tried to combine the results of two techniques, and compare with results of each method being evaluated. Using PCR assay as gold standard, the diagnostic performance of the studied assays revealed that both invasive biopsy-based rapid urease test and *H. pylori* stool antigen test as a non-invasive method had excellent agreement.

Concerning first-choice diagnostic tests for *H. pylori*, the American College of Gastroenterology recommended biopsy-based tests**.** Among these, rapid urease test is the most popular test in clinical practice but has high variable number of false-negative results according to a meta-analysis [42]. False positive results can occur if other urease containing organisms are present in sufficient quantity. The sensitivity of urease test is also affected by the amount of bacteria in the biopsy [43]. In the current study, sensitivity and specificity rates of rapid urease test was 91.7% and 100% respectively detection of *H. pylori* infection from the biopsy site and the pooled 3 biopsies sites helps to detect even more patients with *H. pylori* infection. Our results were comparable with Parihar et al [44] and higher than Allahverdiyev et al [45]. Nevoa et al [46] found that the rate in the detection of *H. pylori* by the molecular method was significantly higher when compared to the rapid urease test.

Several non-invasive tests have been developed to diagnose *H. pylori*. *H.pylori* stool antigen has been detected successfully, using purified antibodies by immunoassay. It is a reliable method to diagnose an active infection and to evaluate the eradication of *H.pylori* infection. However, if the concentration of *H.pylori* antigen becomes low, false negativity may also be reported [47]. In the current study *H. pylori* stool antigen test showed a sensitivity rate of 83.3% and specificity rate of 100%. Higher sensitivity rates (92.4%) and a specificity rate of 100% was reported by Okuda et al [48]. Comparable results were recorded by Me´graud et al [49]. A lower sensitivity rate and a comparable specificity rate were revealed by Korkmaz et al [50]. Saha et al [51] found that *H.pylori*

stool antigen test was superior to upper gastrointestinal endoscopy for detection of *H.pylori* infection and recommended for initial testing for *H.pylori* infection in dyspeptic patients before initiating treatment and before carrying out any invasive procedure such as endoscopy.

In conclusion, *H. pylori* infection rate in Egyptian patients with dyspepsia was high and gastritis was the most revealed finding upon endoscopy. No risk factors were associated with *H. pylori* infection among the studied adult patients. Combined rapid urease and stool antigen tests can be relied upon for detecting *H. pylori* infection. Non-invasive method could be used for *H. pylori* re-testing when invasive tests are negative or could not be done.

Conflict of interest:-
We have no conflict of interest to declare.

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