# Screening of *H. pylori* Positive Patients for the Potential Presence of Atrophic Gastritis through Studying the Gastric Panel and Anti-Helicobacter Antibodies

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***Abstract*—*Helicobacter pylori*,** is a gram-negative bacterium that colonizes the human stomach. Persistent *H. pylori* infection is associated with chronic gastritis, peptic ulcer disease, and in some cases atrophic gastritis, mucosa associated lymphoid tissue (MALT) lymphoma (MALToma), and gastric cancer. Several methods have been employed for the diagnosis of *H. pylori* infection, such as culture, determination of the presence of the microorganism in stained smears and in histological sections. The preformed urease test and PCR which require esophagogastroduodenoscopy to obtain fragments of gastric mucosa in spite of their sensitivity and specificity are not indicated for epidemiological surveys.

The study was conducted to survey the acute and chronic infections with *H. pylori* and their potential association with atrophic gastritis by selecting eighty eight patients, who underwent upper gastrointestinal endoscopy. Patients were grouped according to the results of rapid urease detection kit as *H. pylori* positive group, *n*=44 and *H. pylori* negative group, *n*=44.

Diagnostic markers were assayed for the detection of infection with *H. pylori* with the concomitant atrophic gastritis that are at higher risk for having gastric ulcers and cancer. These markers include anti-helicobacter IgM, IgG, Gastrin 17, Pepsinogen I, and Pepsinogen II. The mean of the patient's age was 39.87+1.50 years with a range of 15-69 years and a mean of age of 38.34$\pm$1.98 was recorded for those who showed urease positivity while urease negativity associated with 41.41$\pm$2.25 mean of age. Males enrolled created 42% of all patients and control while females constituted the rest 58% of individuals recruited. Moreover, it was revealed that out of the 51 females enrolled, only 24 showed urease positivity while 27 were urease negative; on the contrary, males urease testing resulted in 20 and 17 positive and negative reactions, respectively. Thirty six urease negative individuals were reported as *H. pylori* IgG negative whereas 8 patients were equivocal while no case was recorded as positive for *H. pylori* IgG. Of the urease positive figures, 24 samples were reported as *H. pylori* IgG positive while the negative cases were 14 and 6 individuals showed equivocal reaction. Thirty seven urease positive individuals appeared as *H. pylori* IgM negative whereas 3 patients were positive while 4 cases showed equivocal reaction for *H. pylori* IgM. Of the urease negative group, 41 samples were reported as *H. pylori* IgM negative while only one positive case recorded and 2 patients showed equivocal reaction. A significant difference (P <0.001) between urease positive and urease negative groups noticed when their mean absorption values (OD) for *H. pylori* IgG and pepsinogen I antibodies were compared, while all other comparisons between the two groups in relation to their mean absorption values for *H. pylori* IgM, pepsinogen II, and gastrin 17 showed no significant differences.

***Keywords*—*H. pylori*,** Urease, Gastric panel, IgM, IgG

## I. Introduction

*H*E *Helicobacter pylori*, is a gram-negative bacterium that colonizes the human stomach. Persistent *H. pylori* infection is associated with chronic gastritis, peptic ulcer disease, and in some cases atrophic gastritis, mucosaassociated lymphoid tissue (MALT) lymphoma (MALToma), and gastric cancer. [1] About 5% of gastric neoplasms are lymphoid tumors. Nearly all these lesions are now considered to be a consequence of long term *H. pylori* infection[2] . Although *H. pylori* is probably the most common chronic bacterial infection of humans and is present in almost half of the world's population, the exact mode of transmission and natural reservoirs for *H. pylori* are unknown. [3]

Several methods have been employed for the diagnosis of *H. pylori* infection, such as culture [4], determination of the presence of the microorganism in stained smears [5] and in histological sections [6], the preformed urease test [7] and PCR which require esophagogastroduodenoscopy to obtain fragments of gastric mucosa in spite of their sensitivity and specificity are not indicated for epidemiological surveys. [8]

The methods most often used for this purpose are the detection of serum antibodies [9] and the respiratory test with 13C-labelled urea [10]. Among the serologic methods available, ELISA is the most frequently employed because of its speed, simplicity and reproducibility [11].

The study was conducted to survey the acute and chronic infections with *H. pylori* and their potential association with atrophic gastritis.

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## II. Materials and Methods

### A. Patients

A total of 88 individuals (51females and 37 males), aged between 15 and 69 years, were screened for this study. Patients attended the Gastroenterology Unit at AL-Kadhimyia Teaching Hospital in Baghdad from November, 1st 2009 to March 2010 because of complaining from recurrent abdominal pain and other gastrointestinal problems, such as vomiting. All subjects filled out a questionnaire with regard to their general health and were excluded if they had been previously treated for H. pylori infection. The study was approved by the Ethics Committee of the Hospital. After an overnight fast, each patient underwent esophagogastroduodenoscopy, during which three biopsies were taken from multiple sites within the stomach using sterilized biopsy forceps (Olympus 16K; Olympus Corp., Tokyo, Japan).

### B. Rapid urease test

One step H. pylori / Serum / WB RapiCard™ InstaTest (Cat. no. 118562-19-25 CTK Biotech, USA) was used for detection of urease positive samples according to the Instructions of the manufacturer. The test can be interpreted as follows:

Positive: If both the C line and T line appear, the result will indicate that the IgG antibodies specific to H. pylori are detected. A faint line in test region indicates a borderline specimen, which should be re-tested using an alternative method for confirmation.

Negative: If only the C line appears in the control region, the test will indicate that no antibodies to H. pylori are detected.

Invalid: When no control line appears within 5 minutes, the test should be repeated with a new test device.

### C. Blood samples

Serum samples for ELISA assays of IgG and IgM antibodies for H. pylori and serum Gastrin 17, Pepsinogen I, and Pepsinogen II were collected. ELISA kits that had been utilized for this purpose were H. pylori IgM ELISA Test Cat. No. 1504Z, H. pylori IgG ELISA Cat. No. 601 040.03, GASTRIN-17 ADVANCED ELISA Kit for the Measurement of Human Gastrin-17 in EDTA Plasma and Serum Pepsinogen I ELISA Kit for the Measurement of Human Pepsinogen I in EDTA Plasma and Serum Pepsinogen II ELISA Kit for the Measurement of Human Pepsinogen II in EDTA Plasma and Serum; Helsinki/ Finland. The instructions of the manufacturing company were strictly followed when all these kit were put for use.

## III. Results

The gold standard method for stating that the individual had H. pylori infection is urease positivity while those individuals showing negative serum urease test were considered to be uninfected with *H. pylori.* The mean of the patient's age was 39.87 ± 1.50 years with a range of 15-69 years and a mean of age of 38.34 ± 1.98 was recorded for those who showed urease positivity while urease negativity associated with 41.41±2.25 mean of age (Table 1).

TABLE I
MEANS AGE FOR UREASE POSITIVE AND UREASE NEGATIVE INDIVIDUALS

| | POSITIVE UREASE | Negative urease | P |
|---|---|---|---|
| *Means age* | 38.34 ± 1.98 | 41.41 ± 2.25 | 0.310 |

The results of the current study also showed that enrolled males created 42% of all patients and control, while females constituted the rest 58% percentage of individuals recruited for the study (Figure 1). This in turn can be reflected in 1:1.37 male to female ratio.

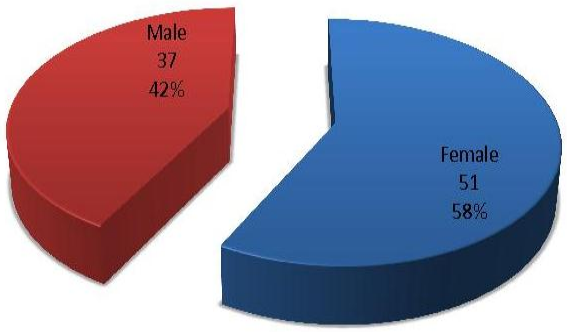


Fig. 1 Female to male ratio for enrolled individuals onlinear data to a higher

Moreover, it was revealed that out of the 51 enrolled females, only 24 showed urease positivity while 27 were urease negative. On the other hand, males urease testing resulted in 20 and 17 positive and negative reactions, respectively (Table 2).

TABLE II
NUMBER OF MALES AND FEMALES RECRUITED AND THEIR RELEVANT UREASE REACTIONS

| Urease test results | Female | Male | Total |
|---|---|---|---|
| *Negative* | 27 | 17 | 44 |
| *Positive* | 24 | 20 | 44 |
| Total | 51 | 37 | 88 |

The results of the present study revealed that 36 urease negative individuals were reported as *H. pylori* IgG negative whereas 8 patients were equivocal, while no case were recorded as positive for *H. pylori* IgG. Of the urease positive cases, 24 samples were reported as *H. pylori* IgG positive,

while the negative cases were 14 and 6 individuals showed equivocal reaction (Table 3).

TABLE III
*H. PYLORI* IGG IN RELATION TO THE UREASE POSITIVE AND NEGATIVE GROUPS

| Urease test results | Equivocal | Negative | Positive | Total |
|---|---|---|---|---|
| *Urease Negative* | 8 | 36 | 0 | 44 |
| *Urease Positive* | 6 | 14 | 24 | 44 |
| Total | 14 | 50 | 24 | 88 |

The results of the present study showed that 37 urease positive individuals appeared as *H. pylori* IgM negative whereas 3 patients were positive, while 4 cases showed equivocal reaction for *H. pylori* IgM. Of the urease negative group, 41 samples were reported as *H. pylori* IgM negative, while only one positive case recorded and 2 patients showed equivocal reaction (Table 4).

TABLE IV
*THE OPTICAL DENSITY OF THE GASTRIC PANEL MAKERS AND ANTI-H.PYLORI IGG AND IGM ANTIBODIES IN UREASE POSITIVE AND UREASE NEGATIVE GROUPS*

| Urease test results | Equivocal | Negative | Positive | Total |
|---|---|---|---|---|
| *Urease Negative* | 2 | 41 | 1 | 44 |
| *Urease Positive* | 4 | 37 | 3 | 44 |
| Total | 6 | 78 | 4 | 88 |

The results revealed that there was a significant differences ($P < 0.001$) between urease positive and urease negative groups when their mean absorption values (OD) for *H. pylori* IgG and pepsinogen I antibodies were compared, while all other comparisons between the two groups in relation to their mean absorption values for *H. pylori* IgM, pepsinogen II, and gastrin 17 showed no significant differences (Table 5).

TABLE IV
*H. PYLORI* IGM IN RELATION TO THE UREASE POSITIVE AND NEGATIVE GROUPS

| Parameter | Urease positive | Urease negative | P |
|---|---|---|---|
| *H. pylori IgG OD** | 1.04 ± 0.28 | 0.74 ± 0.23 | < 0.001 |
| *H. pylori IgG index* | 0.97 ± 0.26 | 0.69 ± 0.21 | < 0.001 |
| *H. pylori IgM OD* | 1.18 ± 0.43 | 1.07 ± 0.41 | 0.213 |
| *H. pylori IgM index* | 0.60 ± 0.22 | 0.55 ± 0.21 | 0.213 |
| *G17OD* | 0.81 ± 0.62 | 0.93 ± 0.79 | 0.432 |
| *Pepsinogen I OD* | 0.42 ± 0.28 | 0.75 ± 0.43 | < 0.001 |
| *Pepsinogen II OD* | 0.25 ± 0.09 | 0.28 ± 0.11 | 0.150 |

*OD: Optical density

## IV. DISCUSSION

Serological testing is a simple noninvasive method for the diagnosis of gastric infections due to *Helicobacter pylori*. It is also well accepted by the patients because of its easiness and noninvasive nature as well as the rapid appearance of results. The importance of serological testing resides within its ability to evaluate the entire stomach accurately. [12]

Many serological kits for *H. pylori* detection are commercially available in clinical practice. The sensitivity of enzyme-linked immunosorbent assay (ELISA)-based serological tests ranges between 90% and 97%, and the specificity ranges between 50% and 96%. [13] The sensitivity and specificity of serological tests mainly depend on the nature of the used antigenic materials.

In addition to the used antigens, the presence of atrophic gastritis is also one of the important factors that influence the test's accuracy. Its reported that a quantitative ELISA test (HEL-pTEST II; AMRAD, Kew,VIC, Australia) had decreased specificity for the detection of *H. pylori* in patients with atrophic gastritis (86.7% vs. 91.9% in patients with and without atrophic gastritis, respectively). However, this test was highly sensitive for the detection of *H. pylori* infection in patients with atrophic gastritis (100.0% vs. 96.5% in patients with and without atrophic gastritis, respectively); therefore, it was concluded that a quantitative ELISA test is suitable for the diagnosis of *H. pylori* infection in patients with atrophic gastritis because of its excellent sensitivity. [14] In patients with atrophic gastritis, all invasive and noninvasive tests for the diagnosis of *H. pylori* infection have their restrictions since the bacterial load of *H. pylori* decreases gradually during the progression of gastric atrophy, and bacteria are unevenly distributed in the stomach. [15]

In addition, the accuracy of serological tests might vary between different races and geographic regions, possibly due to the differences in antigenic properties of local bacterial strains and antibodies of commercial kits used for the diagnosis of *H. pylori* infection. The usefulness of a serological assay should be assessed in a local setting. [16]

The obtained results were inconsistent with that of other's who stated that sero-positivity for *H. pylori* infection was found to be 27.5 % for men and 29.1 % for women. On the contrary, nearly 50 % of the females and 52 % of the males enrolled in the current study tested positive for *H. pylori* infection (indicated by serum urease test). For both studies, the prevalence of *H. pylori* infection and chronic atrophic gastritis were similar between men and women and increased steadily with age (26).

The results proved that there were significant differences between urease positive and urease negative groups when the mean serum levels of IgG and pepsinogen I were compared where these findings were in agreement with previous studies [17, 18]; consequently, atrophic gastritis was more common in helicobacter positive than negative patients in our study group.

Atrophic changes of the corpus can be determined by blood tests, such as low serum pepsinogen I, high serum gastrin, and parietal cell antibodies. [19] Moreover, in a case of advanced atrophy, serology might be the method of choice, since other methods could give false-negative results depending on the density of *H. pylori.* [20]

In line with the obtained results, some researchers recommended the use of one of the invasive test (histology, UBT and culture) in combination with serology for the diagnosis of *H. pylori* infection when atrophic gastritis was considered. [21] The density of *H. pylori* colonization may significantly influence test results, in particular those that are biopsy-based. [22] This may explain the markedly lower sensitivity of biopsy-based tests (RUT, histology, culture) in the presence of atrophy. [23] In contrast, serology is not influenced to such an extent by a lower density of the microorganism, and is reliable even in advanced gastric body atrophy. [24]

It was found that there were no significant differences between serum IgM levels from *H. pylori* infected patients compared with non-infected patients [25] and this finding was consistent with our results. The discrepancies of the results may be attributed to the difference in sample size, follow-up time, and other gastric diseases.

## V. Conclusion

It was concluded that atrophic gastritis is mainly associated with patients who test positive for *H. pylori* infection and that both pepsinogen I can be an excellent biomarker denoting for atrophic gastritis especially in individuals who showed positive results for anti-*H. pyroli* IgG.

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