Serological, Culture and Urea Breath Test for Detection of H. Pylori in Gastric Ulcers Patients

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Abstract

Helicobacter pylori t is the causative agent of duodenal ulcers and gastric ulcers, thus, it is very important to determine the most appropriate system used to diagnose it. The current study use invasive and non-invasive process for diagnosis of *H. pylori*. Culture of *H. pylori* is one of these invasive systems, and the results revealed that 35% of specimen was culture positive (62.5% in male and 37.5 in female) While, the non-invasive method like UBT and ELISA IgG and IgA show 67.1% and 71.5% , 29.2% positive results respectively. The distribution of positive test results between male and female by UBT was (41.3%) male and (58.7%) female) while, the distribution of positive test results between male and female by ELISA IgG and IgA were (61.4%) , (26.3%) in male patients and (78.75%) , (31.25%) in female patients respectively. The results of UBT were increase with age, (34 positive patients in age group 51-60 years old). Serological test of ELISA IgG shows that great % of *H. pylori* infection (74%) found in patients with group age (41-50) yr.O. and the lowest percentage (39%) was found with age group (< 20) years old while, percentages of positive cases of ELISA IgA in young age below 20 years old was 79% related to low % with ages increase.

Key word: *Helicobacter pylori, UBT, H. pylori culture, H. pylori IgA, IgG.*

Introduction

Helicobacter pylori are a spiral-shaped, the persistently Gram-negative bacterium colonizes stomach of the human. It is the essential occasional agent of chronic gastritis superficial and disease ulcer peptic, infection with *H. pylori* is strongly linked with the development of gastric lymphomas and gastric cancer [1].

H. pylori infection needs report as 82% in evolving and 20 to 50% in advanced countries [2]. High propagation of *H. pylori* in some parts of the world is linked, with great happening of severe diseases gastric like peptic ulcers in China (17.2%) or cancer gastric in advanced (61.4%) and evolving countries (64.4%) [3].

Current indications for diagnosis of *H. pylori* infection include non-invasive procedures such as (UBT), fecal *H. pylori* antigen (active infection) and serology (active or prior infections). Invasive tests for *H. pylori* infection include upper gastrointestinal endoscopy, coupled to rapid urease test, culture and histology. Thus, noninvasive systems particularly UBT and tests serological stay the alternate choice of screening the prevalence of *H. pylori* in great populations through epidemiological studies [4].

Methods and Patients

Patients

The study was performed on 137 unselected gastric ulcers **patients (male: 57, 41.6% and female: 80 , 58.4%), aged 15-77 years, who was referred to the endoscopy room of** Disease **Digestive Center in Marjan Medical Hospital, Babylon, Iraq. Biopsy,** Urea breath test and blood sample collection were **done for all patients.**

Urea breath test:

The UBT test is the standard diagnostic tool (Heliforce® - China) and consists of 75 mg ^{13}C-labelled urea in 10 mL of 1.4 g of citric acid solution. Subjects

fasted for at least 2 hours and were free of medication which could influence the UBT results (i.e., of antibiotics in the prior month and no proton pump inhibitors in the previous two weeks) [5]. During the test, the patient swallowed the solution, added on-site to 100 mL tap water. UBT results depend on the gastric hydrolysis of urea by *H. pylori*. In case of infection, $^{13}CO2$ appears in breath, while ammonia is released into the stomach. Breath samples were analyzed by the same ^{13}C-infrared analyzer (HeliFANplus®, FAN GmbH, Leipzig, Germany). The differences between the values at T30 and baseline were expressed as delta over baseline (DOB, δ‰), with a normal cut-off up to 4‰. As recommended by the manufacturer, the DOB at T30 was the reference value.

Procedure:

1) Subjects fasted for at least 2 hours before the examination. 2) Slowly exhale into the first air bag and make it full of gas as much as possible. Immediately fasten the lid of air bag tightly. This one is the base line bag (T=0). 3) Dissolve the urea [^{13}C] granules in 80-100 ml cooled water, drink it up and set still for 30 minute. 4) When time up, collect the second breath sample (T=30) like step tow. 5) Test the tow sample bags with the IR-FORCE infrared Spectrometer.6) Use δ‰ (millesimal difference) to determine the result. Δ‰ is define as :

$$\delta‰ = \frac{\text{13C - isotopic abundance of 30 min. sample} - \text{13C - isotopic abundance of 0 min. sample}}{\text{13C - isotopic abundance of 0 min. sample}}$$

Culturing of *H. pylori*:

Two samples of antral biopsy are acquired for each patient. One for histological examination and the second of culture ,examination smear and put in sterile semi solid transport media (bacteriological agar 0.16 %, NaCl 0.9 %) and transported to the laboratory of microbiology before 2 hr. **Antral biopsies was inoculated onto surface of selective Brucella blood agar including** defibrinated **7% of the blood sheep , trimethoprim (5 ppm),vancomycein (5 ppm), amphotericin B (4 ppm) and polymyxin B (50 µg/L) . Cultured plates was incubated at 37 ℃ under conditions microaerobic for rise humidity cultures was examined after 5-7 days of incubetion of observing pinpoint glistening colonies. Cultures Negative was furthermore incubated and spotted upward to two weeks. A bacterial strain was identified as *H. pylori* on the basis of Gram stain and spiral microscopic semblance as well as activities positive of oxidase** catalase and **urease [6].**

Serological tests:

ELISA IgG and IgA kits (Monobind Inc., USA) were used to analyze each serum samples following the instruction of manufacture supplied by kits.

Ethical approval:

The base ethical approval of the Digestive Disease Center in Marjan Medical Hospital, Babylon, Iraq was obtained. Furthermore, all collected patients of the work was knowledge and the approval was acquired of each one before the aggregate the samples.

Result and Discussion

Urea Breath Test:

Out of 137 patients with peptic ulcer, 92 (67.1 %) were positive for UBT, 38 (41.3%) male and 54 (58.7%) female (Table-1). This result agreement with results obtained by Rotem *et al,* [7] who found that *H. pylori* positive result via UBT is more in female (60.7 %) than male, while Eisdorfer *et al,* [8] found that the prevalence of *H. pylori* contagion was like women and men, 51.5% vs 52.9% .

Table-1: UBT positive and sex distributions

Methods	sex groups	
	Male	Female
UBT	38 (41.3%)	54 (58.7%)
Total	92 (100%)	

In this study the UBT was increase with age, (34 positive patients in age group 51-60 years old) as shown in figure-1. These results were come in line with result obtained by Rotem *et al,* [7] but differ from results obtained by Zevit *et al,* [9] who found that UBT positive result of *H. pylori* was increase with decrease age. This difference may by beyond to deference in geographic region of study and socioeconomic state for people associated in these study.

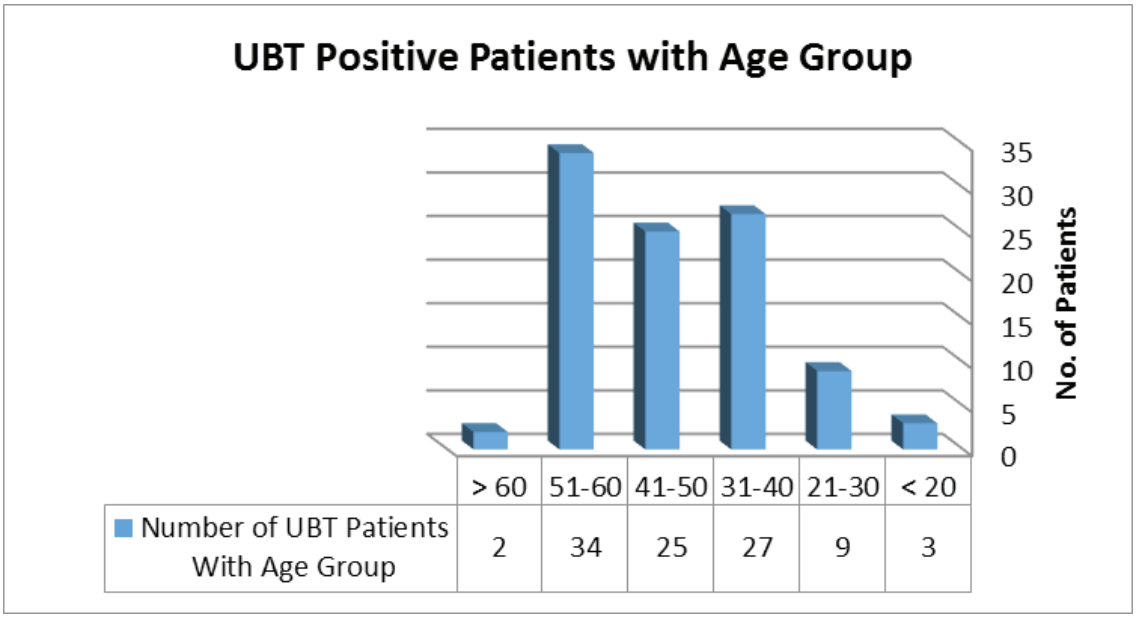

Figure-1: Correlation between positive UBT and age.

Culture and ELISA IgG & IgA for detection of *H. Pylori*:

Two separate ELISA kits (IgG and IgA) were utilized of detection of *H. pylori* infection in 137 patients and utilizing a culture system for demonstrated *H. pylori* in biopsies. The microaerophilic organism was identified in 35% of Culture (62.5% of positive samples were recorded in males compared with 35.5% in females) as illustrated in Table-2.

Table-2: Percentage and distribution of *H. pylori* culture with sex.

Methods	With H.pylori/ Number of patients %		Without H.pylori/ Number of patients %	
Culture	Male	Female	Male	Female
	30 / 62.5	18 / 37.5	27 / 30.3	62 / 69.7
Total	48 / 35		89 / 65	

This findings were in line with those reported by other researchers [11] who observed that *H. Pylori* positive culture effects are observed in (37.2%) of patients.

While, *H.pylori* was observed via ELISA IgG and IgA in 71.5% and 29.2% of patients at the same order, appear Table-3.

Table-3: Serological tests and cultural systems utilized for detection of *H. pylori*.

Test	Positive for H. pylori		Negative for H. pylori	
	NO.	Percentage	NO.	Percentage
IgG	98	71.5	39	28.5
IgA	40	29.2	97	70.8

ELISA IgG was agreed by [11,12] that ELISA IgG were found to be 73.9 % and 73 % respectively in patients with *H. Pylori* infection, while those results were in conflict with the results of [13] which found only 32.3%. The causes of these dissimilar outcomes may be due to the geographical dissemination of the *H. pylori* infection and stage of infection. However, ELISA IgA was same to those found by [12], that found patients 25% was positive of *H. pylori*, and dissimilar to those found via [11,13], who found 82.3% and 58.2% respectively. The similar factors mentioned in ELISA IgG can be attributed to this dissimilarity in the results.

The result of ELISA IgG shows that great % of *H. pylori* contagion (74%) found patients with group age (41-50) years old whereas, the lowermost percentage (39%) found with group age (< 20) years old appear figure-2. The significant correlation among ages and positive *H. pylori* cases with ELISA IgG in positive cases increase with age increase. These result is like they found via the study of [12-14] who found A major impact of age on *H. pylori* positive cases in which anti- *H. pylori* IgG antibodies have been increased with ages increased, whereas The results distressed with results [15] who found no association among age and seropositivity of *H. pylori*. This may be due to that risk of infection which tends to increase with age or due to the iron and vitamin deficiency are decrease in elderly that lead to increase the *H. pylori* infection in this group as demonstrated by [16,17].

High percentages of positive cases reported by ELISA IgA were 79% in people under the age of 20 years compared to percentages low with ages increasing; as appear in fig. 2. But there is no significant correlation among age groups, *H. pylori* infection via ELISA IgA test. These results were in disagreement with those of [12], found that *H. pylori* IgA seropositivity increased with age.

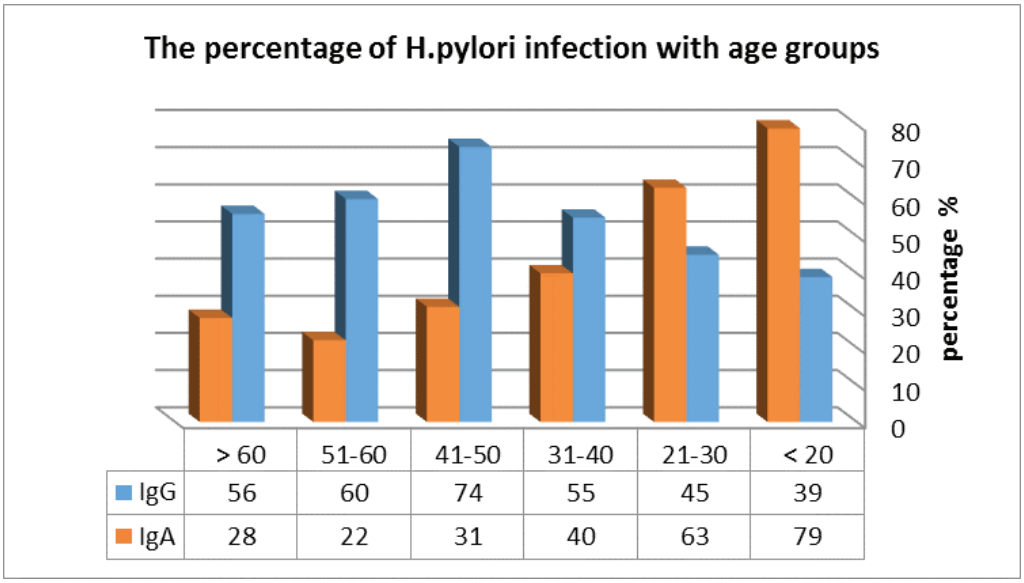

Figure-2: Percentages of *H. pylori* infection in age groups utilizer techniques ELISA.

H. pylori detected 35 (61.4%) and 15 (26.3%) in male patients via ELISA IgG and IgA at the same order. While, in female patients, *H. pylori* detected 63 (78.75%) and 25 (31.25%) via ELISA IgG and IgA at the same order, appear in the Figure-3.

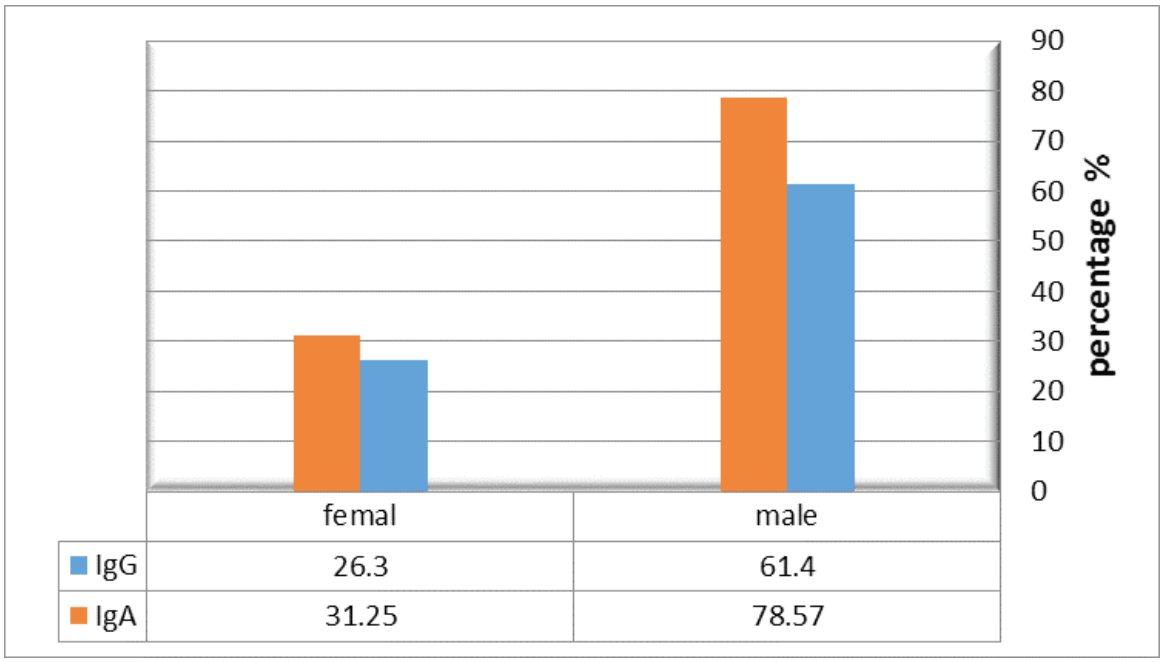

Figure-3: Percentages of *H. pylori* infection in 6 groups via ELISA IgG and IgA tests.

This study reached that there is no significant correlation among sex and *H. pylori* screening methods of ELISA (IgG and IgA). These findings was parallel to results obtained by [18,19] for IgG , [13] for IgA, while difference of the [20] found the males was most infected than females via ELISA IgG and IgA.

Conclusion

Considered the UBT is one the utmost essential and reliable noninvasive process of detection of *H.pylori*; UBT 13C is suitable for epidemiologecal study of the adults and children , particularly when endoscopy is not strictly necessary; ELISA IgG was superior to ELISA IgA for the detection of anti *H. pylori* in adults patients; and Found among age and *H. pylori* infection via ELISA IgG in which *H. Pylori* contagion increase with the age increase.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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