

ISSN: 1991-8941

## Immune and non-immune diagnosis of *H. pylori* In Patients with dyspepsia.

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### Abstract:

**Background:** The roles that T helper type 1 (Th1) specific immune responses in protection from *H. pylori* challenge was understood. It is expected that Th2 immune responses are required for protection against extracellular bacteria, such as *H. pylori*. Both invasive and non-invasive tests are used in the diagnosis of *Helicobacter pylori* infection. The non-invasive tests avoid endoscopy and encompass the serologic and breath tests. This study aimed to show important immune and non-immune tests for *Helicobacter pylori* infection diagnosis in patients. **Patients and Methods:** A total of one hundred seven (107) adult patients from both genders were attending Gastro Endoscopy Unit at Ramadi Teaching Hospital to undergo selective OGD from December 2012 to May 2013. Multiple mucosal biopsy specimens were taken for rapid urease test (RUT) to detect *Helicobacter pylori* in tissue samples. After endoscopy, blood specimen was taken from each patient to be used for serological tests including; IgG, IgM, by ELISA. Rapid Chromatographic Immunoassay test (CAS) was used for IgG against *H. pylori* also. **Results:** Present study showed that the rate of infection in males was same as in females, and increased within age group (31-50) years old, it was found that higher positive results of CAS, and RUT for *H. pylori*, especially in younger adults. Findings confirmed that a significant relationship between *H. pylori* rapid urease test (RUT) with IgG and IgM specific for *H. pylori* antigen.

**Key Words:** Immune , non-immune , diagnosis , *H. pylori* , dyspepsia

### Introduction :

*Helicobacter pylori* is the most important etiological factor responsible for chronic gastritis, duodenal ulcer, gastric ulcer [Sainz et al, 1999 ; Muller et al, 2007]. The microorganism resist local host defense mechanisms through its the ability to withstand acidic gastric pH and its motility. [Israel & Peek, 2001]. The presence of a potent urease sets it apart from other oxidase- and catalase-positive bacilli. The enzyme urease metabolizes urea to carbon dioxide and ammonia to buffer the gastric acid [Kuwahara et al, 2000]. Chronic gastritis induced by *Helicobacter pylori* increases the risk for a wide spectrum of clinical outcomes, ranging from peptic ulcer disease (gastric and duodenal ulceration) to distal gastric adenocarcinoma and gastric mucosal lymph proliferative diseases, such as non-Hodgkin's lymphoma [Parsonnet et al, 1994; Alevizos et al, 2012]. *Helicobacter pylori* can be transmitted oral-oral and fecal-oral, it has been detected in dental plaque, saliva and feces [Kabir, 2003]. The organisms can be cultured from vomitus or diarrhoeal stools, suggesting the potential for transmission among family members during periods of illness [Me'graud & Lehours, 2007]. *Helicobacter pylori* has been

demonstrated worldwide and in individuals of all ages. Estimates suggested that 50% of the world's population is affected. Infection is more frequent and acquired at an earlier age in developing countries compared to industrialized nations [Coh et al, 2011]. Both invasive and non-invasive tests are used in the diagnosis of *Helicobacter pylori* infection. The non-invasive tests avoid endoscopy and encompass the serologic and breath tests [Tetterman & Morris, 2014]. The Rapid urease test is one of the invasive tests. It is based on the principle that abundant urease enzyme produced by *Helicobacter pylori* hydrolyses urea to ammonia. The consequent rise in the pH of the medium is detected by phenol red indicator [Berry & Sagar, 2006]. It is suggested and found to be more sensitive in some studies in comparison to biopsy histology. Gastric imprint smears stained with Gunwald-Gens method is a rapid and cost effective method in addition to histology for detecting *H. pylori* in patients undergoing upper gastrointestinal endoscopy and biopsy [Rahbar et al, 2012]. Bacterial culture and sensitivity testing, String test [Leong et al, 2003], Brushing urease test [Vlaichone et al, 2002]. A variety of non-invasive tests for the diagnosis of *Helicobacter pylori* are available

or being evaluated. These include: [Lopes et al, 2014], Urea breath test (UBT) [Gatta et al, 2004], serology [Feldman et al, 1995], 13C bicarbonate assay [Gisbert & Pajares, 2004] stool antigen [Braden et al, 2000], salivary [Fallone et al, 1996] and urinary assays [Kato et al, 2000]. Serology rely on the concept that Infection by *Helicobacter pylori* induces a both local and systemic antibody response. The typical pattern is that of a transient increase of IgM followed by an increase of the IgA and IgG levels that persists throughout the infection. [Akhiani et al, 2005]. In this study, both important immune and non-immune tests for *H. pylori* infection diagnosis in patients were studied.

#### **Patients and methods :**

A total of (107) adult patients from both genders were attending Endoscopy Unit at Ramadi Teaching Hospital to undergo elective Esophagogastroduodenoscopy (OGD) from December 2012 to May 2013. They were suffering from dyspepsia. The clinical diagnosis of patients was performed by Senior gastroenterologist. Patients were excluded from the study if they were:

1. Taking a proton pump inhibitors .
2. Taking H2-blockers .
3. *H. Pylori* inhibiting antibiotic
4. Presence of active bleeding peptic ulcer.

According to the exclusion criteria, a total of 107 patients examined in the Gastro Endoscopy Unit within age range between (18-75 years).

#### **Specimens collection:**

##### **A: Biopsy Specimens :**

Each biopsy specimen was placed in urea medium for rapid urease test. Plastic slides were incubated at room temperature (15-30°C) aerobically, and observed for 15-20 minutes and again at one, three, and six hours of incubation for the development of a pink-red or red-violet color. Negative specimens were reincubated for up to 20 hours. [Aydin et al., 2004].

##### **B: Blood Specimens :**

After endoscopy, blood specimen was taken from each patient. (3ml) of venous blood was collected using sterile disposable syringe and the serum was pooled from each blood specimen by centrifugation for 3 min at 3000rpm. Serum samples were kept frozen at (-20 °C) to be used for serological tests.

#### **Serological tests :**

##### ***Helicobacter pylori* Ab Rapid Cassette test (CAS):**

*Helicobacter pylori* Ab Rapid test is a sandwich lateral flow Chromatographic

immunoassay for the qualitative screening detection of antibodies (IgG) anti-*Helicobacter pylori* in human serum. This test was done using (ASANSouth Korea, Rapid Urease Kit )

##### **ELISA Test for IgG and IgM:**

ELISA test was used for IgG IgM specific for *H. pylori* using special DRG (USA) Elisa kit for each test. Methods for Elisa test were followed as described by manufacturer company.

##### **Statistical Analysis :**

All data were analyzed using the SPSS statistical program (Statistical Package for the Social Science) Version 14.0. Statistical significance was taken with P value <0.005. The significant differences were detected by using either the Goodness fit test within Chi-square test or independent sample t-test.

#### **Results :**

##### **Patients and their grouping:**

A total of (107) patients; (53 females and 54 males ), with age range was (18-75). Lowest number of Patients was within the age groups (51-70) and above 70 years (Table -1).

##### **Rapid Urease Test (RUT):**

Positive result of urease test showed pink color in the presence of *Helicobacter pylori*. During one minute to 1 hour, seventy three specimens 73(68.2) were showing positive result. (Fig- 1).

##### **Chromatographic Immunoassay (CAS):**

Sixty four 64 (59.8%) patients were showing positive (CAS) test in serum for *Helicobacter pylori* (Table -2 ).

##### ***Helicobacter pylori* IgG using ELISA Test:**

Positive IgG specific for *H.pylori* was detected in 102 (95.33%) serum samples (Fig.2) All cases which were showing positive IgGs showed positive urease test. *Helicobacter pylori* positive patients showed significantly ( $p<0.001$ ) higher titers of anti *Helicobacter pylori* IgG ( $1.840 \pm 0.421$ ) in serum samples than *Helicobacter pylori* negative individuals. there was a significant relationship between results of tests for *H pylori* infection, (IgG CAS, & RUT) .

##### ***Helicobacter pylori* IgM**

Only five (5) (4.7%) patients were showing positive IgM against *Helicobacter pylori* by ELISA method (Fig-2).

#### **Discussion :**

Out of (107) patients under investigations results in (Table - 1) showed that rate of infection in males was same as in females, there was no statistically significant difference between genders ( $p=0.163$ ). This agreed with other studies done by: Chen et al [2014] in USA, Zhang et al [2014] in China, Formichella et al [2013] in Germany and Bures et al [2012]

in Czeck. *H. pylori* seropositivity rate among the general population varies in the different regions and age groups in the world. (Table -2) showed that the rate of infection in this study undergo increase within age group (31-50) years old, this agreed with Vilaichone et al [2013] who found the more likely of infection in patients under 50 years old (76%) than in older patients (24%), another group of Iraqi researches Al-Marsoumy & Jabbo [2013], found that (79.2%) of patients in Baghdad area were under 50 years, and Hasan [2011] from Erbil detected that increase of infection in ages less than 50 years (76%). It was recognized that prevalence of *H. pylori* infection increase with psychosomatic. [Rosenstock, et al 1996] *H. pylori* infection was assessed by serology, Immunoglobulin G (IgG) antibody against *H. pylori* by using the ELISA method (Enzyme-linked immunosorbent assay), which are considered non invasive gold standard methods by McNulty et al. [1999], and recommended by Kienesberger et al. [2012] which was 100% specific and 93% sensitive. A negative value in RUT depend on non homogeneous distribution of the microorganism in the stomach and this situation is overcome by use of several specimen from (3-5) for the same patient. [Quintana-Guzmán et al, 1999; Lim et al, 2004]. So we minimize the specimen error and this explain the 2 (1.9%) patients which gave negative result by this method, which lowering the percentage of infection comparing with other methods. Chromatographic Immunoassay CAS result for 11(10.3%) patients who were showing false negative, which have positive result in ELISA method, this was might be due to production of low detectable circulating antibody response [Austarheim et al, 2013]. The presence of specific *H. pylori*-directed IgG antibodies has shown excellent correlation with the presence of *H. pylori* enteric infection this was in accordance with the findings of [Megraud&Lehours, 2007]. The sensitivity of biopsy urease tests is about 90 to 95 percent, and specificity is 95 to 100 percent and false positive tests are unusual. These findings were also confirmed by [Howden& Hunt 1998].

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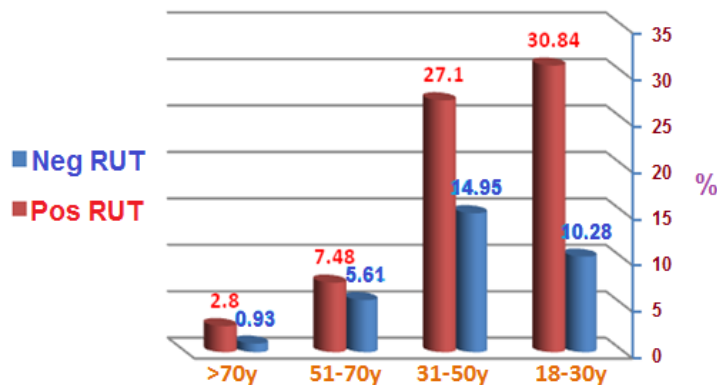
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**Table (.1): Gender and age groups of patients .**

Sex	age groups								Total	
	18-30		31-50		51-70		> 70			
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Female	24	22.43%	24	22.43%	3	2.80%	2	1.87%	53	49.53%
Male	20	18.69%	21	19.63%	11	10.28%	2	1.87%	54	50.47%
Total	44	41.12%	45	42.06%	14	13.08%	4	3.74%	107	100.00%

**(Table .2): H.Pylori Casette positive and negative test in patients.**

H.Pylori CAS	age groups								Total	
	18-30		31-50		51-70		>70			
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Negative	21	19.63%	17	15.89%	4	3.74%	1	0.93%	43	40.19%
Positive	23	21.50%	28	26.17%	10	9.35%	3	2.80%	64	59.81%
Total	44	41.12%	45	42.06%	14	13.08%	4	3.74%	107	100.00%



**Fig. ( 1 ): Distribution of Helicobacter pylori positive and negative Rapid Urease .**

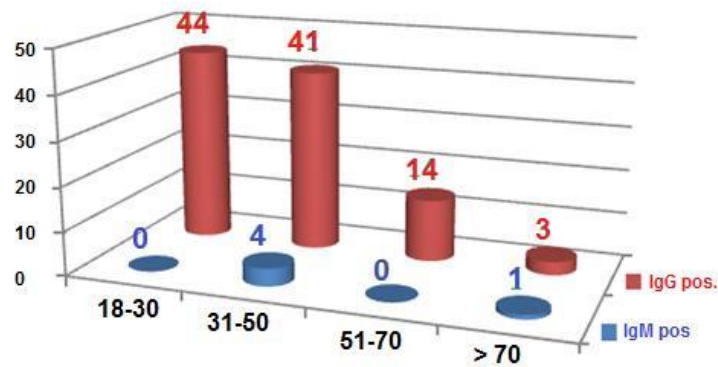


Fig (2): Distribution of ELISA: IgM positive and IgG positive results

التشخيص المناعي وغير المناعي لجرثومة الملويات البوابية في مرضى الذين يعانون من سوء الهضم  
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الخلاصة:

ان دور الخلايا التائية نوع الاول في الدفاع المناعي ضد جرثومة اللوبيات البابية معروف جيدا ويعتقد ان دور الخلايا التائية النوع الثاني مطلوب لنفس المهمة المذكورة اعلاه. ان كل من الطريقة المؤلمة وغير المؤلمة مستخدمة في تشخيص الإصابة بجرثومة الملويات البوابية. تشمل طريقة التشخيص غير المؤلمة تجنب طريقة ناظور المعدة واتباع طريقة اختبار النفس والطرائق السيرولوجية. هذه الدراسة لبيان الطرائق المناعية وغير المناعية في التشخيص المرضى وطرائق العمل : تضمنت هذه الدراسة ( ١٠٧ ) مريضا من الذكور والاناث راجعوا شعبة الناضور المعدي مستشفى الرمادي التعليمي للفترة من كانون الثاني ٢٠١٢ ولغاية ايار ٢٠١٣ ليخضعوا لفحص ناظور المعدي . اخذت عدة خزعات نسيجية من كل مريض لفحصها باختبار اليوريا السريع لفحص وجود جرثومة اللوبيات البابية فيها . بعد اختبار الناظور اخذت عينة دم ( ٣ مل ) من كل مريض لعزل مصل الدم لاستخدامه لاختبار الكروماتوغرافي السريع والاختبارات السيرولوجية الاخرى للكشف عن وجود الأضداد ( IgM ، IgG ) الخاص بجرثومة اللوبيات البابية بطريقة الاليزا. النتائج: بينت الدراسة ان نسبة الإصابة في الذكور مشابهة لتلك في الاناث وان اعلى نسبة اصابة كانت ضمن الاعمار ( ٣١-٥٠ ) سنة . لوحظ ايضا ان نسبة الاختبارات الموجبة لاختبار اليوريا السريع واختبار الكروماتوغرافي اكثر من النتائج السالبة سيما في الاشخاص الاقل عمرا . وتبين ايضا هناك ترابط بين نتائج اختبار اليوريز السريع واختبار الأضداد ( IgM ، IgG ) الخاص بجرثومة اللوبيات البابية بطريقة الاليزا.