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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 thathigher positive results of CAS, and RUT for *H. pylori*, especially in younger adults. Findings confirmed that a significant relations hip between *H. pylori* rapid urease test (RUT) with IgG and IgM specific for *H. pylori* antigen.

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 [Goh 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 [Testerman& Morris, 2014]. The Rapid

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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 Grunwald-Giemsa 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 [Vilaichone 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]. Inthis 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 selective 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^{O}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 ^{0}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).

EISA 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 s howing pos itive(CAS) test in serum for *Helicobacter pylori* (Table -2).

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

PositiveIgG specific for *H.pylori* was detected in 102 (95.33%) s erum s amples (Fig.2) All cas es which were showing positive IgGs howed

pos itive ureas e tes t. *Helicobacter pylori* pos itive patients showed significantly ($p<0.001$) higher titers of anti *Helicobacter pylori* IgG (1.840 ± 0.421) in s erum s amples than *Helicobacter pylori* negative individuals .there was a significant relations hip between res ults of tes ts for *H pylori* infection, (IgG, CAS, & RUT) .

***Helicobacter pylori* IgM**:

Only five (5) (4.7%) patients were s howing pos itive IgM agains t *Helicobacter pylori* by ELISA method (Fig-2).

Discussion :

Out of (107) patients under inves tigations res ults in (Table - 1) s howed that rate of infection in males was s ame as in females , there was no s tatis tically s ignificant difference between genders (p=0.163). This agreed with other s tudies 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* s eropos itivity rate among the general population varies in the different regions and age groups in the world. (Table -2) s howed that the rate of infection in this s tudy undergo increas e 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%), ano ther group of Iraqi res earches Al-Mars oumiy & Jabbo [2013], found that (79.2%) of patients in Baghdad area were under 50 years , and Has an [2011] from Erbil detected that increas e of infection in age les s than 50 years (76%). It was recognized that prevalence of *H. pylori* infection increas e withps ychos omatic. [Ros ens tock, et al. 1996].

.*H. pylori* infection was as s es s ed by s erology, Immunoglobulin G (IgG) antibody against *H. pylori* by us ing the ELISA method (Enzyme - linked immunos orbent as s ay), which are cons idered non invas ive gold s tandard methods by McNulty et al. [1999], and recommended by Kienes berger et al. [2012] which was 100% s pecific and 93% s ens itive. A negative value in RUT depend on non homogeneous dis tribution of the microorganis m in the s tomach an d this s ituation is overcomed by us e of s everal s pecimen from (3-5) for the s ame patient. [Quintana-Guzmánc et al, 1999; Lim et al, 2004]. So we minimize the s pecimen error and this explain the 2 (1.9%) patients which gave negative res ult by this method, which lowering the percentage of infection comparing with other methods . Chromatographic Immunoas s ay CAS res ult for 11(10.3%) patients who were s howing fals e negative, which have pos itive res ult in ELISA method, this was might be due to production of low detectable circulating antibody res pons e [Aus tarheim et al., 2013]. The pres ence of s pecific *H. pylori*- directed IgG antibodies has s hown excellent correlation with the pres ence of *H. pylori* enteric infection this was in accordance with the findings of [Megraud&Lehours , 2007]. The s ens itivity of biops y ureas e tes ts is about 90 to 95 percent, and s pecificity is 95 to 100 percent and fals e pos itive tes ts are unusual. Thes e findings were als o confirmed by [Howden& Hunt 1998].

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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 Cassette 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%

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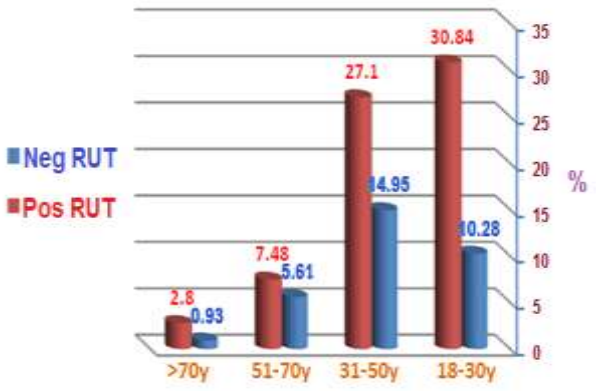
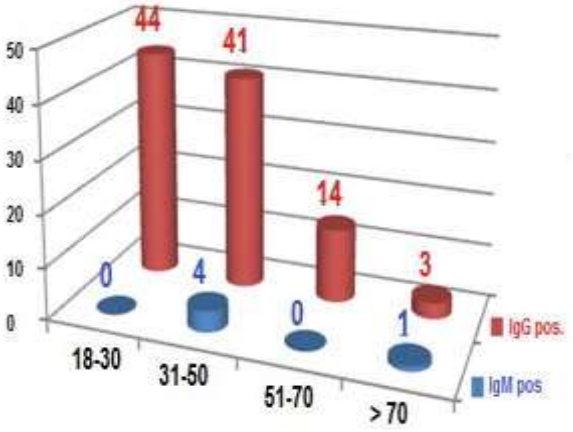


Fig. (1): Distribution of Helicobacter pylori positive and negative Rapid Urease.



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

اسيل قاسم حسين شهاب احمد لافي ياسين حمد مجيد

الخلاصة:

ان دور الخلايا الثايمية نوع الاول في الدفاع المناعي ضد جرثومة اللوبيات البابية معروف جيدا ويعتقد ان دور الخلايا الثايمية النوع الثاني مطلوب لنفس المهمة المذكورة اعلاه. ان كل من الطريقة المؤلمة وغير المؤلمة مستخدمة فى تشخيص الاصابة 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 فى التشخيص المرضى وطرائق العمل : تضمنت هذه الدراسة (107) مريضا من الذكور والاناث راجعوا شعبة الناضور المعدي مستشفى الرمادي التعليمي للفترة من كانون الثاني 2012 ولغاية مايس 2013 ليخضعوا لفحص ناظور المعدي . اخذت عدة خزعات نسيجية من كل مرض لفحصها باختبار اليوريا السريع لفحص وجود جرثومة اللوبيات البابية فيها . بعد اختبار الناظور اخذت عينة دم (3 مل) من كل مريض لعزل مصل الدم لاستخدامه لاختبار الكروماتوغرافى السريع والاختبارات السيرولوجية الاخرى للكشف عن وجود الأضداد (IgM ، IgG) الخاص بجرثومة اللوبيات البابية بطريقة الاليزا.النتائج:بينت الدراسةان نسبة الاصابة في الذكور مشابهة لتلك في الاناث وان اعلى نسبة اصابة كانت ضمن الاعمار (50−31) سنة . لوحظ ايضا ان نسبة الاختبارات الموجبة لاختبار اليوريا السريع واختبار الكروماتوغرافى اكثرمن ا لنتائج السالبة سيما في الاشخاص الاقل عمرا .و تبين ايضا هناك ترابط بين نتائج اختبار اليوريز السريع واختبار الاضداد (IgM ، IgG) الخاص بجرثومة اللوبيات البابية بطريقة الاليزا.