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

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Abstract:
Background: The role that T helper type I (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 specimens were taken from each patient to be used for serological tests including: IgG, IgM, by ELISA. Rapid Chromatographic Immunoassay tests (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 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 bacteria. 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 [Paris et al. 1994; Alevi et al. 2012]. Helicobacter pylori can be transmitted oraloral and fecal-oral, it has been detected in dental plaque, saliva and feces [Kabir, 2003]. The organisms can be cultured from vomitus or diarrheal stools, suggesting the potential for transmission among family members during periods of illness [Mcgraud & Lehors, 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 urease test is one of the invasive tests. It is based on the principle that abundant urease enzyme produced by Helicobacter pylori hydrolyzes 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 Gurrwall-Gens a 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. 2012], Brushing urease test [Vachon 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 [Fekihm et al. 1995], 13C bicarbonate assay [Grb et al. & 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. [Akhimi 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 endoscopy and take biopsies from the gastric antrum. They were suffering from dyspepsia. The clinical diagnosis of patients was performed by a 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 (26°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. [Ayden 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.

Sero logical tests:
Helicobacter pylori Ab Rapid Cassette test (CAS):
Helicobacter pylori Ab Rapid test is a sandwich lateral flow Chromatographic: immunoas say for the qualitative screening detection of antibodies (IgG) anti-Helicobacter pylori in human serum. This test was done using (ASANSouth Korea, Rapid Urease Kit).

HSA Test for IgG and IgM:
ELISA test was used for IgG, IgM specific for H. pylori using special DRG (USA). 1 test kit for each test. Methods for HSA 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 Immunoas say (CAS):
Sixty four (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 were positive. IgG showed positive urease test. Helicobacter pylori positive patients showed significantly (p<0.001) higher titers of anti Helicobacter pylori IgG (1.840 ± 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 Czech. *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 Vakichone 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-Maroumy & 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 age less than 50 years (76%). It was recognized that prevalence of *H. pylori* infection increase with psychosomatic. [Rosens tock, 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 Keresberger 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; Lm 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 Immunosay CAS result for 11(10.3%) patients who were showing false negative, which have positive result in ELISA method, this might be due to production of low detectable circulating antibody response [Austenhein 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 test 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].

**References:**


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

<table>
<thead>
<tr>
<th>Sex</th>
<th>18-30</th>
<th>31-50</th>
<th>51-70</th>
<th>&gt;70</th>
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<tr>
<td></td>
<td>No</td>
<td>(%)</td>
<td>No</td>
<td>(%)</td>
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</tr>
<tr>
<td>Female</td>
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<td>22.43%</td>
<td>24</td>
<td>22.43%</td>
<td>3</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>18.69%</td>
<td>21</td>
<td>19.63%</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>41.12%</td>
<td>45</td>
<td>42.06%</td>
<td>14</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>H.Pylori CAS</th>
<th>18-30</th>
<th>31-50</th>
<th>51-70</th>
<th>&gt;70</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>(%)</td>
<td>No</td>
<td>(%)</td>
<td>No</td>
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<tr>
<td>Negative</td>
<td>21</td>
<td>19.63%</td>
<td>17</td>
<td>15.89%</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>21.50%</td>
<td>28</td>
<td>26.17%</td>
<td>10</td>
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<tr>
<td>Total</td>
<td>44</td>
<td>41.12%</td>
<td>45</td>
<td>42.06%</td>
<td>14</td>
</tr>
</tbody>
</table>

Fig. (1): Distribution of Helicobacter pylori positive and negative Rapid Urease.
التشخيص المناعي وغير المناعي لجرثومة الملوليات البوابية في مرضى الذين يعانون من سوء الهضم

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الخلاصة:

ان دور الخلايا الثايمية نوع الأول في الدفاع المناعي ضد جرثومة اللوبيات البابية معروف جيداً ويعتقد ان دور الخلايا الثايمية النوع الثاني مطلوب لنفس المهمة المذكورة أعلاه. ان كل من الطريقة المؤلمة وغير المؤلمة مستخدمة في تشخيص الاصابة بجرثومة الملوليات البوابية. تشمل طريقة التشخيص غير المؤلمة تجنب طريقة ناظور المعدة واتباع طريقة اختبار النفس والطريقة السيرولوجية. هذه الدراسة لبيان الطريقة المناعية وغير المناعية في التشخيص اليوس وطرق العمل: تضمنت هذه الدراسة (172) مريضاً من الذكور والأول اتادوا شعبة الدراسات المعاييري مستشفى الرمادي التعليمي للمفردى من كانون الثاني 2012 ولغاية مايو 2013 للاختبار لتشخيص الاصابة بجرثومة اللوبيات البوابية. اختصت عدة خرائط نسيجية من كل مريض لفحصها باختبار اليوز السريع لتقصب وجود جرثومة الملوليات البوابية فيها. بعد اختبار الناظور اختخت عينة دم (3 مل) من كل مريض لعزل مصل الدم لإختبار الكروماتوغرافى السريع، واختبارات السيرولوجية الأخرى للكشف عن وجود الاستثمار البوابية بطرق الاليزرا. النتائج: بينت الدراسة نسبة الاصابة في الذكور مشابهة للكثير في الانته ين اقح نسبة اصابة كانت ضمن الاعمار (31-50) سنة، وظف أيضاً أن نسبة الاختبارات الموجبة لاختبار اليوز السريع واختبارات الكروماتوغرافى اكتربوا انتواج (IgM، IgG) بالسالبة، اما في الاشخاص الأقل عمراً، وثيب أيضاً هناك ترابط بين نتائج اختبار اليوز السريع واختبار الاضداد (IgM، IgG) الخاص بجرثومة الملوليات البوابية بطرق الاليزرا.