Endoscopic screening and Serological diagnosis of Helicobacter pylori among symptomatic individuals

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ABSTRACT
Recently the opportunity of peptic ulcer or gastric infection has been increased and dispersed in the world and caused by H. pylori. Many medical diagnoses have been used to detect this pathogenic bacterium and were performed by invasive and non-invasive methods. Towards this end, the study address here evaluating the performance of the endoscopic diagnosis for gastroduodenal part from digestive tract of suspected individuals, serological diagnosis for those who had a history of medication, seroprevalence of Helicobacter pylori infection among suspected patients, and assessment of the correlation of demographic factors with carrier cases in Duhok city. ELISA technique was used for the detection of anti-Helicobacter-specific IgG, IgM, and IgA antibodies. A structured
questionnaire interview for each patient was performed to investigate the endoscopic examination, record of information about occupation, and history of treatment associated with *H. pylori* infection. Endoscopy technique used as a nonsurgical procedure to examine a patient's internal tract or organ such as gastroduodenal tract, unlike many other medical techniques, endoscopy is inserted directly into the organ without opening the body.

Out of 86 tested samples, *H. pylori* were found in 71%, 30%, and 6% by ELISA IgG, IgA, and IgM. According to the occupation factor, the housewife group had higher percentages of IgG than those affiliated with the military group which was recorded a lower percentage of IgG. A significant relationship was found between the student's group and seropositive IgG by ELISA and also a significant correlation was detected between the housewife group and the seropositive IgM. A higher percentage of IgG were found among subjects with no history of medication than who have a history of medication. There was a positive association between those who have not taken antibiotics and the positive case of IgG by ELISA. The highest percentage of antral gastritis was recorded by IgG among endoscopic findings while in the case of Normal endoscopy lowest percentage was recorded. A significant correlation was between seropositive IgG and antral gastritis. In the conclusion, *H. pylori* have wide prevalent in the housewife group which requires high awareness to reduce exposure to *H. pylori* subsequently to decrease possibilities of transmission. There was a statistical correlation between antral gastritis and serological IgG by ELISA method.

1. INTRODUCTION

*Helicobacter pylorus* is a helical–curved, flagellated, and gram-negative bacterium that colonizes the human stomach. The microaerophilic environment is the main requirement for the growth of this pathogenic bacterium. Currently, many endoscopic findings have been
related significantly to this pathogenic bacterium such as chronic ulcerative, gastric and duodenal metaplasia, dyspeptic symptoms, and severe gastroduodenitis. The prevalence of this microorganism varies among different countries and different populations. Approximately, about 30% to 50% is prevalent among children and reaches 90% during adulthood in developing countries while 50% in developed countries [1]. This bacterium was firstly discovered by Robin Warren and Barry Marshall in 1983 who reported the presence of Helicobacter pylori in the mucosal layer of the stomach thereafter has been an isolated microorganism from gastric biopsy called it Campylobacter pyloridis, and reclassified as Helicobacter pylori. Recently, great challenges have occurred in the medicament planning of peptic ulcer treatment. prior to this event microbiologists and infectious disease specialists believed the stomach was empty from any kind of microorganism due to overproduction of acid which causes gastritis, therefore, their treatments were towards suppressing acid only. After confirmation by several studies, it has been enhanced a fact that this bacterium is the essential reason for peptic ulcers and is related to gastric carcinoma. Consequently, the strategy of medication was changed from anti-suppressing drugs to both antibacterial and anti-acid drugs [2]. Many risk factors that contribute to the spreading of H. pylori which is including poor sanitation, overcrowding of areas, and low education levels [3]. Many authors have asserted the transmission of this pathogenic microbe is contagious which is done by oral – oral and fecal-oral ways [4]. Pains in the upper gastric region, dyspepsia, hematemesis, and melena have been the commonest clinical characteristics for endoscopy examination. Endoscopic diagnosis has been used to screen the endoscopic parameters in the whole parts of the stomach and duodenum and a lot of biopsy samples were needed from the mucosal layer of gastroduodenal and diagnosed by Histological examination, culture, rapid urease test as an invasive method whereas Serological detection of H.pylori was performed for identifying different antibodies against H.pylori in the suspected individual’s serum by using Enzyme-Linked Immunosorbent Assay, Rapid Diagnostic Test, Immunochromatography (ICT) as a non-invasive method [5]. The strategy of using drug treatment could reduce the possibility of the pathogenicity progression of H.pylori and subsequently risk factors associated with gastroduodenitis and gastroduodenal cancer [6], according to their pathogenicity, this colonize microorganism can alter the microenvironment of the stomach by producing the urease enzyme which makes facilitate colonization as well. Many biological mediator substances appear to be a role play in adherence of this microorganism to the mucosal layer of the gastric part of the digestive tract which includes cell surface cell surface glycolipid, adhesins proteins, and others bio-mediators such as Cecropins has been regarded as a competitive factor for inhibiting the growth of other microorganisms [7]. Impaired host immunity could increase the rate of infection of H.pylori pathogenic bacterium and this agent is revert to the cancer (especially acute lymphocytic leukemia), Human immunodeficiency virus, Iron deficiency, chronic diarrhea and malnutrition [8]. In reverse to other pathogenic bacteria that are highly clonal (such as Shigella dysenteriae and Mycobacterium tuberculosis), and it has been proposed a lack of clonality resulting it was genetic heterogeneous. The heterogenetic structure of H pylori is probably an adaptation of this bacterium to the gastroduodenal inflammation of its host [9]. In spite of the fact magnification of endoscopy provides more accurate information in concern to the unusual mucosal layers parameters; it is not available in all units of endoscopy. Furthermore, high training and experience are required to use this medical nonsurgical technique by specialized endoscopy or supervisor of endoscopy have expertise. In addition, endoscopy is not necessarily suitable for routine clinical practice because it is time-consuming and uncomfortable for the patients. [10].
2. LITERATURE REVIEW

Since the discovery of this infectious pathogen, especially in the recent decade, \textit{H. pylori} was regarded as the most important agent that causes mucosal inflammation in the deep stomach layer and can be detected by numerous medical laboratory tests. These tests may be biopsy depend called invasive and independent biopsy called non-invasive method [11].

For performing biopsy-dependent method gastroscopy is the main requirement to obtain a biopsy from suspected patients for diagnosis of microorganism by histopathological examination, isolation & culture, Urease tests, in addition to molecular diagnosis such as Polymerase chain reaction. While in the case of independent biopsy method several ways have been used which include Urease breath test, Sero-diagnosis, Stool antigen test [12]. Previously several studies have focused on the seroprevalence of different age categories among them children and adults consequently high increasing rates of gastritis and duodenitis have been recorded due to patchy distribution of this bacterium among different populations and its association with geographical areas [13]. The exact transmission mode of this pathogenic microbe is still unclear but directly from one suspected individual to another (oral/oral and fecal/oral) was regarded as the main route of transmission through different ways which involve contaminated water, food, and use of contaminated endoscopic tube from an infected person and reused to an uninfected person[14]. \textit{H. pylori} was localized in the mucosal layer of the stomach without clinical characteristics of most people; therefore, there is insufficient therapy for the eradication of this pathogenic bacterium and this reason reverts to a strong association between \textit{H. pylori} and inflammation of the mucosal layer of the gastroduodenal part of the digestive tract [15]. Various researches have demonstrated that the diseases and clinical manifestations were related to \textit{H. pylori} and it was the main reason for stomach disorders such as gastric ulcer, gastroduodenal metaplasia, gastroesophageal reflux disease (GERD) and duodenal ulcer. Many signs and symptomatic manifestations associated with this epidemiologic disorder were revealed such as discomfort sensation, abdominal pain, nausea, and vomiting [16]. \textit{H. pylori} was detected by [17] about 37.9% of Immunoglobulin IgG in the serum sample of patients and the diagnostic method was performed by the ELISA kit. ELISA technique was used by [18] and found 14 positive cases of IgG and 16 positive cases of IgA. [19] Obtained Three cutoff points 3.3UI/ML of IgA, 6.4 UI/ML of IgM, and 9.9UI/ML of IgG by using the ROC-analysis method. Enzyme-Linked Fluorescent Assay (ELFA) technique was used by [20] to detect seroreactive cases among 68 patients and indicated the higher concentrations of IgG and IgA of symptomatic patients in comparison to asymptomatic cases with significant correlation in comparison to the titer of IgM with no statistical association.

3. METHODS AND MATERIALS

3.1. Study design
The cross-sectional study design was carried to achieve the aims of the present study.

3.2. Specimens collection and methodology
about 5ml of blood samples were taken under sterile environmental conditions then separated into serum by centrifuge technique and placed into storage freezer until reached to the process of serological diagnosis using Anti-Helicobacter pylori IgG, IgM and IgA antibodies ((Monobind Inc., USA)) ELISA test kits.

3.3. Endoscopic findings
The endoscopy technique was conducted under an Endoscopy specialist and general anesthesia. After the examination process, a conclusion report was written and saves in the clinical investigation document.
3.4. Data collection
The conducting of the present study was done in the Azadi hospital/endoscopy center and all referred suspected patients were recorded in their interview form. Questionnaire form and conclusion report of endoscopy specialist from each patient were filled which included socio-demographic data: occupation factor, history of treatment, and endoscopic examination.

3.5. Statistically analysis
SPSS (Statistical package for social sciences) software version 25 was used to find statistically analysis of the present data and the probability value or P-value (ordinarily ≤ 0.05) is regarded as significant.

4. RESULTS

A total of 86 suspected patients were enrolled in the current study. Of these, about 71% of IgG, 6% of IgM, and 30% of IgA were seropositive cases by using the ELISA method as shown in Table 1.

<table>
<thead>
<tr>
<th>Serological tests</th>
<th>Seropositive of H. pylori</th>
<th>Seronegative of H. Pylori</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>IgG</td>
<td>61</td>
<td>71</td>
</tr>
<tr>
<td>IgM</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>IgA</td>
<td>26</td>
<td>30</td>
</tr>
</tbody>
</table>

In accordance with occupation status, the housewives group recorded the highest positive rate among those who had performed other occupational works and the military group indicated the lowest rate. A significant relationship was found between the student's group and seropositive IgG by ELISA (P = 0.038) and also a significant correlation was detected between the housewife group and the seropositive IgM (P = 0.042) as shown in Table 2.
Table 2. Prevalence of Anti-\textit{Helicobacter pylori} IgM, IgG and IgA Seropositivity According to occupation status

<table>
<thead>
<tr>
<th>Occupation</th>
<th>No. of P.</th>
<th>IgG Positive</th>
<th>IgM Positive</th>
<th>IgA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Housewives</td>
<td>32</td>
<td>24</td>
<td>75%</td>
<td>3</td>
</tr>
<tr>
<td>Government jobs</td>
<td>8</td>
<td>7</td>
<td>87%</td>
<td>0</td>
</tr>
<tr>
<td>Private jobs</td>
<td>16</td>
<td>14</td>
<td>87%</td>
<td>0</td>
</tr>
<tr>
<td>Retired</td>
<td>11</td>
<td>8</td>
<td>72%</td>
<td>0</td>
</tr>
<tr>
<td>Students</td>
<td>12</td>
<td>7</td>
<td>58%</td>
<td>1</td>
</tr>
<tr>
<td>Military</td>
<td>7</td>
<td>1</td>
<td>14%</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>5</td>
<td>6%</td>
<td>61</td>
</tr>
</tbody>
</table>

Table 3. Reveals the effect of medication on suspected individuals that have had a history of therapy and how it is associated with \textit{Helicobacter pylori} infection as detected by ELISA. According to the present study, the rate of those who were not taking medication was higher than those who admitted medication by the ELISA IgG, IgM, and IgA as a diagnostic method. A significant association was found between those who have not had a history of treatment and the positive case of IgG by ELISA (\textit{P}.Value = 0.0006)

Table 3. Prevalence of Anti-\textit{Helicobacter pylori} IgM, IgG and IgA Seropositivity According to history of therapy

<table>
<thead>
<tr>
<th>History of Therapy</th>
<th>No.</th>
<th>Immunoreactive IgM</th>
<th>Immunoreactive IgG</th>
<th>Immunoreactive IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Medication</td>
<td>33</td>
<td>2</td>
<td>6%</td>
<td>24</td>
</tr>
<tr>
<td>No medication</td>
<td>53</td>
<td>3</td>
<td>5%</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>5</td>
<td>6%</td>
<td>61</td>
</tr>
</tbody>
</table>

Among endoscopic parameters, antral gastritis was extremely an active inflammation in the mucosal layer of the stomach in comparison to the other characteristics of gastroduodenal findings. Based on the current study outcomes was observed a considerable association between antral gastritis and seroreactivity case of IgG (\textit{P} < 0.01), as shown in table 4.
Table 4. Prevalence of Anti-Helicobacter pylori IgM, IgG and IgA Seropositivity According to endoscopic findings

<table>
<thead>
<tr>
<th>Endoscopic findings</th>
<th>No. of P.</th>
<th>IgG Positive</th>
<th>IgM Positive</th>
<th>IgA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Normal OGD</td>
<td>32</td>
<td>5</td>
<td>42%</td>
<td>0</td>
</tr>
<tr>
<td>GERD</td>
<td>8</td>
<td>4</td>
<td>80%</td>
<td>0</td>
</tr>
<tr>
<td>Antral gastritis</td>
<td>16</td>
<td>30</td>
<td>73%</td>
<td>4</td>
</tr>
<tr>
<td>Duodenitis</td>
<td>11</td>
<td>10</td>
<td>77%</td>
<td>1</td>
</tr>
<tr>
<td>Gasteroduodenitis</td>
<td>12</td>
<td>12</td>
<td>80%</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>61</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

5. DISCUSSION

In the last decade, Helicobacter pylori conquered the world in different populations because of the recording excessive rate in the number of infections. Originally Helicobacter pylori were responsible for causing much gastroduodenal pathologic disorder and different endoscopic findings parameters. Many symptomatic contagions have been noted and associated with this microbe [21].

In the current study from 86 cases: 6%, 30%, 71% were recorded as a seroreactive suspected specimens of IgM, IgA, and IgG respectively by a semi-automated technique called ELISA. The present study outcomes were acceptable to those found via [22] who detected 66.13% of serum Anti-Helicobacter pylori IgG and 12% of IgM by ELISA method and [23] found the highest serum level of IgG about (88.86 ± 42.0) Au/ml and (2.09 ± 1.58) Au/ml of IgA in patients who suffered from antral and duodenal ulcerative with the recording of significant correlation between endoscopic antral gastritis and serum titer of IgG by ELISA method. Seroreactive outcome in the current study was similar to the study performed by [24] who indicated the serum titer of IgG (72%) and IgM (36%) by Enzyme-Linked Immunosorbent Assay with noted of significant correlation between Serum level of IgG against H. pylori and gastritis. Whereas the present study outcomes did not correspond with other studies such as [25] who found a high proportion of anti-Helicobacter IgM (46.9%), IgA (43.5%), and IgG (41.9%) by serum ELISA technique. These inconsistencies revert to many reasons including different phases of infection; most ELISA methods provide an inconsistent result, impairment of mucosal defense mechanism against H. pylori, number of samples, genetic susceptibility.

Based on the current study findings, the highest percentage of seropositive cases of the housewives group were observed with statically significant with ELISA IgM while the lowest rate of seropositive samples of the military group with a significant relationship with ELISA IgG was found. This outcome is similar to the proposed data found by [26] that observed 49.6% of seropositive samples of housewives workers from 152 dyspeptic patients by using serodiagnostic and found a static correlation with H. pylori. And also it is in correspond to the related study by [27] who detected seroreactive cases about 57.7% among individuals with dyspepsia symptom and revealed there was considerable relation between H. pylori seroreactive and occupational housewives factor. The current study findings were disagreed with those detected by [28] and found about 28.3% of serum anti-H. pylori IgG among symptomatic patients and no notable association has been found between this bacterium infection and housewives worker. These dissimilarities are based on many agents such as the area of the study in addition to socioeconomic differences, low number of samples, handling with the contaminated cooking instrument. In the current findings, the rate of seroreactive
serum IgG level with this bacterium among the students' group was crucially increased and observed notable association between this pathogenic microbe and students and this outcome agreement with others such as [29] who found 55% of serum titer of IgG antibody by using ELISA technique. Significantly, it was found to be correlated. The seroprevalence rate of this study is contradictory with the findings detected by [30] who detected 67.6% of students were seropositive by ELISA method and revealed there were no statistically correlation differences between the seropositive of *H.pylori* infection and students group based on the occupation factor. Close contact in the overcrowded area among students such as schools, game parks, and ecological factors like poor environmental hygiene and genetic predisposition to *H. pylori* infection could be probable factors that contribute to increasing the rate of infection of this pathogenic bacterium. The host immune response is not an adequate defensive mechanism to eradicate this pathogenic bacterium because many influential agents that could affect the host immune responses consequently there was the spectacular challenge of management of this pathogenic microbe; recently this strategy has been changed by medication from acid-suppressing drugs into antacids and antibiotics. The decreasing of opportunity of infection in this study was found on those who have had a history of therapy reverse to those who have not had a prior of treatment with notable association to those who have not had. The current data was identical to [31] who found consumption of a nonsteroidal anti-inflammatory (NSAID) and the antimicrobial drug could contribute to reducing the possible infection of *H.pylori* and also detected there was an association of NSAID ingestion and seropositive cases.

6. CONCLUSION

A considerable high percentage of women workers in house areas are susceptible to infection by *H.pylori* representing a serious public health risk. Not all endoscopic manifestations have an association with *H.pylori* infection because the present study found no static correlation with endoscopic findings except antral gastritis and not all clear gastroduodenal tract means there is no infection or no clinical condition associated with *H.pylori* because the present study found the Normal OGD (Oesophago-gastro-duodenal) had positive cases. Unfortunately, those who have not had previous medication have an association with *H.pylori* and the present study found medication can reduce the rate of infection among symptomatic individuals.

7. RECOMMENDATION

ELISA IgG test could be used as a diagnostic method for the identification of *H. pylori*. Plenty of specimens should be included in future studies to get more precise results. Furthermore, an extra field study is needed to better interpretation between *Helicobacter pylori* and demographic characteristics by using a validated diagnostic test for *H. pylori*.

REFERENCE


