THE RELATIONSHIP BETWEEN IMMUNOGLOBULIN’S (IGA,IGE) AND PATIENTS WITH HELICOBACTER PYLORI

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ABSTRACT

*H. pylori* is one of the most common pathogens in the world. Where our study included its effect on the body’s immunity, especially immunoglobulin (IgA, IgE) and the extent of this immunoglobulin change by infection, and this study was conducted at the University of Babylon, Faculty of Science, Department of Biology. The study included 71 patients and 50 control samples, in which samples (blood, biopsy, and stool) were collected from each patient and observer. The work was carried out in the Teaching Hospital of Gastroenterology and Hepatology in the Medical City of Baghdad and the General Hospital of Al-Suwaira in Wasit Governorate for the period from September 2020 to April 2021. The aim of the study was to measure (IgA, IgE) The affected subjects and their comparison with healthy subjects by the method of ELISA, as well as the relationship of these criteria with age and sex. Where the results showed a significant increase in the values of (IgA, IgE) in the blood of patients compared with healthy subjects. The statistical data also showed an increase in the percentage of (IgA, IgE) in women compared to men.

I. INTRODUCTION

*Helicobacter pylori* is a Gram-negative anaerobic conditions bacteria that colonizes the human stomach and causes chronic gastritis, peptic ulcer adenocarcinoma, and mucosa associated lymphoid tissue lymphoma. This disease is thought to infect around half of the world's population (Zamani et al., 2018). *H. pylori* are small and 0.5-2 mm in diameter. In modern culture bacteria appear in the form of a straight rod with an irregular curved rod, while in ancient culture they appear to be spherical in shape (Kimang’a et al., 2010; Sofroniew, Howe, and Mobley, 2001). Infection is thought to play an important role in stomach cancer diseases which is one of the most common malignancies with an estimated 1 million cases worldwide in 2012 (Park et al., 2018). However, over 80 percent of individuals infected with this bacterium are asymptomatic (Hamzah and Aljanaby, 2020). *H. Pylori* infection may be associated with the induction of various autoimmune disorders such as idiopathic rheumatoid arthritis Thrombocytopenic purpura (also called immune thrombocytopenic purpura) (Tanoue et al., 2019). *H. pylori* is capable of producing a large amount of urease in the stomach, which protects against gastric acidity. Urease breaks down urea to create NH3 and CO2, as well as ammonia, which disturbs the epithelium. Ammonia interacts with neutrophil metabolites, causing harmful agents to develop and thereby increasing the risk of stomach cancer. In addition, urease stimulates the synthesis of inflammatory cytokines (Olive et al., 2014). The ability of primary bacteria to colonize gastrointestinal mucus is dependent on a number of characteristics, including the release of the bacterial urease enzyme (to resist acidic environment in the intestinal mucosa), and the presence of polar flagella (for locomotion) can successfully pass the stomach mucosa barrier and reach critical epithelial cells, as well as alterations in bacterial cell shape. Environmental factors, host factors (host gene polymorphism), and bacterial virulence factors such as the (Cytotoxin-associated gene A) (CagA) and the vacuolating toxin (VacA) in the epithelium may all contribute to *H. pylori* colonization and disease exposure (Karkhah et al., 2019). The stomach bacteria *H. pylori* has been identified as a group 1A carcinogen of gastrointestinal malignancies due to its increased production of superoxide radicals and nitric oxide (Uemura et al., 2001). The production of a number of pro-inflammatory cytokines, including interleukin-8 (IL-8) and tumor necrosis factor (TNF-), is assumed to be implicated in the development of *H. pylori*-induced gastric inflammation in gastric mucosal infection with *H. pylori*. Many inflammatory disorders are caused by the cytokine family IL-17, which includes IL-17A, B, C, D, E (also known as IL-25), and F. The most well-studied is IL-17A, which is largely produced by T helper type 17 (Th17) cells. IL-17A appears to have a key role in the path physiology of *H. pylori*-associated illness, according to several lines of evidence (Dai et al., 2020). Inflammatory cytokines...
released in response to *H. pylori* infection cause the recruitment and growth of antigen-sensitive T lymphocytes and B cells that generate immunoglobulins’ (IgA and IgG). However, unless antibiotic treatment is given, the immune response will not be able to eradicate the germs, and the infection will usually continue a lifetime (Ihan, Pinchuk; Beswick, 2012).

II. MATERIALS AND METHODS

Collection of samples and processing

This study included a total of 121 individuals with gastrointestinal problems. The results of the tests based on a rapid antibody (blood) test were for everyone, individuals who gave a positive result were considered sick, and people who gave a negative result were considered a control group. Also, 60 stool samples were obtained from sixty people from the same group, and they were also divided into patients whose test result was positive and (the control group) whose test result was negative.

Blood samples

Three (3) ml of blood samples was collected from each patient and control, put in gel tube and preserved in safety cool box, transported through two hours to microbiology lab, centrifugation and then divided to two parts, first part for rapid *H. pylori* diagnosis test and second part was used to determination of IgA, IgE, by ELISA methods.

Antibody rapid test

The *H. pylori* serum antibody fast test was developed on the basis of a qualitative membrane immunoassay for detecting *H. pylori* antibodies in whole blood, serum, or plasma. Anti-human IgG is immobilized in the test line region of the test in this test. When the sample is placed in the device's sample well, it reacts with the *H. pylori* antigen coated particles in the test. This mixture migrates along the length of the test chromatographically and interacts with the immobilized anti-human IgG. A colored line will emerge in the test line region if the samples contains *H. pylori* antibodies. The test is done to evaluate which bacteria are present in blood samples. In the absent of infection with the bacterium, the red color appears only on the letter C (control line), however in the presence of bacteria, a red package will appear on the letter T (result line) in addition to the red packaging control, as illustrated in Figure (1).

Figure (1) : Antibody rapid test

Stool samples

Stool antigen rapid test

The test is used to diagnose the bacteria in stool samples. Small samples of stool specimens were obtained from three separate regions of the stool specimen, passed to a vial containing diluents, forcefully shaken, and dropped roughly two to three drops into the round window of the test cassette after two minutes of resting the tube. After 10 minutes of incubation at room temperature, two colored lines, C (control) and T (test), appeared across the center window of the cassette, indicating a positive test, while only one line in C indicated a negative result. A light-colored line in the letter T was also regarded as favorable (Silva et al., 2010). as it is shown in Figure (2).
Culturing of *H. pylori*

The gastric biopsy specimens were conveyed to the microbiological laboratory in 2-2.5 mL brain heart infusion broth as a liquid suspension in less than two hours. Incubated for 24 hours at 37°C, then subculture for 3-5 days on modified blood agar base and skirrow agar.

Cluster of differentiation (IgA and IgE) assay procedure

1. All chemicals, stock solution, and samples were made exactly as directed. Before use, the reagents were recorded at room temperature. At room temperature, the examination will take place.

2. Working out how many strips are needed for the assay. For use, strips have been put into the frames. Keeping leftover strips around 2-8 °C is a good idea.

3. The standard well received 50 μl of the standard well. Because the standard solution contains an antibiotic, the antibody is not added to the standard well.

4. A 40 μl sample was added to the well sample, followed by 10 μl of IgA antibody, 50 μl of Streptavidin-HRP, and finally 50 μl of Streptavidin-HRP to the well and standard well samples (not a good blank control). It was thoroughly mixed. Sealant was applied to the plate. Incubate for 60 minutes at 37 degrees Celsius.

5. After removing the sealant, the board was cleaned five times with washing solution. For each wash, soak wells for 30 seconds to 1 minute with at least 0.35ml of washing solution. All wells with laundry storage are washed and cleaned five times for automatic washing, and wells are filled with laundry storage. Use a tissue or another absorbent item to clean the bandage.

6. Each well received 50 μl of platform A liquid, followed by 50μl of platform B solution. Incubate plate covered with fresh sealant in the dark for 10 minutes at 37 °C.

7. Poured 50 μl of suspension solution into each well; the blue color will quickly turn yellow.

8. Within 10 minutes of adding the stop solution, determining the optical density (OD value) of each well using a microplate reader set to 450 nm.
The micro-plate reader must be opened in advance at 450 nm for the user, the device is preheated, and the test parameters set, as shown in Figure (3).

III. RESULTS

Results and distribution of sample collection

This study included a total of 121 individuals suffering from digestive problems. The results of the tests, based on the rapid antibody test for all individuals, were 71 patients who gave a positive result, and 50 gave a negative result, they were considered a control group. Also, 60 stool samples were obtained from sixty people of the same group, and the result of their examination based on the Stool antigen rapid test was 40 patients and 20 gave a negative result (control) were collected directly from people in the Endoscopy Unit of the Medical City Hospital for the Digestive System, the Hospital for Hepatology and the General Hospital in Al-Suwaira for the period from September 2020 to April 2021. The data collected includes name, age and gender stool as shown in Table (1).

Table (1): Distribution of sample numbers according to the type of sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total</th>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>121</td>
<td>71</td>
<td>50</td>
</tr>
<tr>
<td>Stool</td>
<td>60 from 121</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Biopsy</td>
<td>60 from 121</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

Biopsy culture

In our study, 60 samples were cultured from a total of 60 individuals. The result was identical to Stool Antigen Diagnostic Test, as 40 samples gave a positive result and 20 samples gave a negative result, as shown in Table (2). Stomach biopsy samples were taken from the injured and those samples were transferred by means of a transporter such as 0.9% physiological saline, 20% w/v glucose (Morgan et al., 1990), and brain heart infusion broth BHI in a cooled box and within a period of time less than three hours to the laboratory for the purpose of cultivation (Ndip et al., 2003).

Stool culture

Based on the results of the rapid screening test (rapid stool antigen test), 40 samples were diagnosed that gave a positive result and 20 samples gave a negative result (the control group) out of a total of 60 samples from the same individuals 121. The positive and negative samples were cultured on special culture media for H. pylori such as (skirrow medium and modified blood agar). As demonstrated in Table 2, 15 (25%) of the infected

Figure (3): This standard curve(IgA,IgE)
samples were positive in the case of culture, but the negative samples (control group) did not yield a positive result on the stool. This study was similar to the study (Parsonnet et al., 1999, Dore et al., 2000). As their study showed percentages of 21% and 25% respectively. The growth of \textit{H. pylori} bacteria appeared on the culture media used in the form of small, circular colonies and has a transparent cream color, as shown in Figure (4) and these characteristics were similar to the characteristics mentioned by (Xia, Keane and O’Morain, 1994).

Table (2): Biopsy growth results and stool culture

<table>
<thead>
<tr>
<th>Sample</th>
<th>Biopsy culture</th>
<th>Result culture</th>
<th>Stool culture</th>
<th>Result culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>40</td>
<td>60</td>
<td>15</td>
</tr>
</tbody>
</table>

Sensitivity 100% 100%
Specificity 100% 25%

Figure (4): \textit{H. pylori} characterization

\textit{H. pylori} colonies on modified blood agar base after 5 days incubation period in anaerobic condition. Small, entered white to creamy colonies
Quantitative ELIZA test of IgA and IgE in patients compared with control

The ELIZA score for IgA and IgE recorded a significant increase in patients compared to control samples (blood) samples. The IgA score was recorded, 92.59 ± 42.17 in the patient group versus 78.50 ± 18.70 in the control group, (P < 0.01). The IgE results also recorded a clear increase, as it was 325.73 ± 127.37 in the patient group compared to 285.98 ± 67.48 (P-value = 0.00) in the control group, as illustrated in Table (3).

Table 3: Concentration of IgA and IgE- ELIZA( ng/ml) in blood of patients compared with control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient</th>
<th>Control</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>92.59±42.17</td>
<td>78.50±18.70</td>
<td>0.01</td>
</tr>
<tr>
<td>IgE</td>
<td>325.73±127.37</td>
<td>285.98±67.84</td>
<td>0.00</td>
</tr>
</tbody>
</table>

IgA and IgE Concentration in Patients and Control According to the Gender

The results showed a significant increase in female IgA and IgE in patients compared to controls, while there was no increase in the results of blood samples in males, the result of blood samples (IgA) was 83.38 ± 15.86 in male patients compared to 85.90 ± 9.53 in a control for males and 97.92 ± 51.41 female patients compared to 73.95 ± 21.70 in the female control (P.value = 0.03). While the (IgE) samples were 278.59 ± 28.66 in the male patients compared to 306.16 ± 17.97 from the controls and 353.01 ± 153.42 in the female patients compared with the control 273.56 ± 83.93 from the female controls (P. value = 0.02) as indicated in Table (4).

Table 4: Concentration of IgA and IgE ELIZA ( ng/ml)in blood of patients compared with controls according to the Gender

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>Patient</th>
<th>Control</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>Male</td>
<td>83.38±15.86</td>
<td>85.90±9.53</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>97.92±51.41</td>
<td>73.95±21.70</td>
<td></td>
</tr>
<tr>
<td>IgE</td>
<td>Male</td>
<td>278.59±28.66</td>
<td>306.16±17.97</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>353.01±153.42</td>
<td>273.56±83.93</td>
<td></td>
</tr>
</tbody>
</table>

The concentration of IgA and IgE in patients and control according to age groups

The results recorded a significant increase in IgA and IgE for patients compared to controls in the age groups (less than 20 years and more than 45 years) in (blood) samples, except for the second age groups (21–45 years), where there was a decrease in the results of (IgA,IgE) for blood samples as shown in Table No (5).

Table 5: Concentration of IgA and IgE ELIZA ( ng/ml)in blood of patients compared with controls according to the age groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age (years)</th>
<th>Patient</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>Less than 20</td>
<td>113.18±60.61</td>
<td>77.47±21.44</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>21-45</td>
<td>80.97±27.19</td>
<td>87.87±19.76</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>More than 45</td>
<td>86.84±31.90</td>
<td>70.16±11.44</td>
<td>0.01</td>
</tr>
<tr>
<td>IgE</td>
<td>Less than 20</td>
<td>359.97±185.08</td>
<td>260.94±53.26</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>21-45</td>
<td>305.90±83.62</td>
<td>333.62±85.38</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>More than 45</td>
<td>316.72±110.98</td>
<td>263.37±35.24</td>
<td>0.00</td>
</tr>
</tbody>
</table>
IV. DISCUSSION

Through the tests that were used in this study, the diagnosis of stomach biopsies gave the best results and were identical to the results of the stool test as shown in the table (1). This study agreed to consider endoscopy and biopsy to assess infection with H. pylori through histology and rapid urea culture testing and culture as a gold standard in the Diagnostic Algorithm Study (Gisbert and Abraira, 2006). In this study, a significant increase was observed in the special statistical data (IgA, IgE) shown in Table No(2). In general, it may be associated with H. pylori infection, and this result is similar to the study (Surawut et al., 2018). The data also showed that the concentration of (IgA, IgE) in the blood showed different elevations in the three age groups. Where the highest increase was observed in the first age group (less than 20 years) and a lower rate of increase was observed than in the third age group (greater than 45 years). As for the second age group (21 to 45 years), there was no increase in the level of (IgA, IgE), and these results were similar. For the results of (El-Maksoud et al., 2019), except for the second group, there was a slight increase. Furthermore, it has been observed that the direct engagement of extracellular toll-like receptors (TLR2) on B1 type cells with H. pylori urease stimulates the synthesis of different auto antibodies without the involvement of T cells. This could explain the link between H. pylori infection and autoimmune disorders. As a result, it’s been suggested that H. pylori components like urease are to blame Components of H. pylori, such as urease, may be among the environmental triggers that induce autoimmune disorders by rousing B1 cells and promoting the generation of auto antibodies (Jafarzadeh et al., 2013).

REFERENCES