The Role of Helicobacter pylori in Induction of Gastric Autoimmunity

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Abstract—Introduction: Helicobacter pylori (H. pylori) is a member of the causative factors of digestive system complaints, such as gastritis, stomach ulcers, mucosal-associated lymphoid tissue lymphoma (MALT), and gastric cancer (GC). This research aimed to determine the H. pylori bacteria's possible roles in autoimmune gastritis (AG) and assess the sera concentrations of vitamin B₁₂ (VB₁₂) and iron in individuals with and without H. pylori disease. Methods: In the Province of Thi-Qar, Iraq, case-control research was conducted during the time of November-2021 to June-2022, which included 55 subjects having H. pylori-associated diseases and 35 healthy individuals. The eligibility criteria of both study groups were followed stringently. For the patient's group, subjects underwent an endoscopic examination and a stool antigen test (SAT). Additionally, the subjects of all research cohorts were assessed for their sera levels of anti-H. Pylori-antibodies (Abs), parietal cells auto-Abs (PCA), VB₁₂, and iron. Results: The findings revealed a significantly higher level of PCA positivity and lower levels of VB₁₂ and iron by comparing the patient group with the control group. In both study groups, serum level of anti-H. pylori immunoglobulin gamma (IgG) Abs positively associated with PCA and inversely correlated with serum VB₁₂ and iron in the patient's group. Conclusions: There were correlations between H. pylori infection and PCA-positive status, lower VB₁₂, and low iron levels. Anti-H. pylori-IgG serum concentration was a valuable biomarker for the risk of developing AG, low VB₁₂, and iron levels. Parietal cells auto-Abs were not associated with VB₁₂ and iron levels, and the two former biomarkers were not correlated.

Keywords: H. pylori, Autoimmune gastritis, Vitamin B₁₂, Iron.

Abbreviated:
Abs, antibodies; Ag, antigen; AIG, autoimmune gastritis; ATPase, adenosine triphosphatase; dl, deciliter; ELISA, enzyme-linked immunosorbent assay; g, gram; H. pylori, Helicobacter pylori; HC, healthy control; HPP, H. pylori-associated patients; IDA, iron deficiency anemia; IF, intrinsic factor; IgG, immunoglobulin gamma; L, liter; µg, micrograms; MALT, mucosal-associated lymphoid tissue lymphoma; ml, milliliter; PA, pernicious anemia; PCA, parietal cells auto-Abs; pg, picograms; pmol, picomoles; SAT, stool antigen test; U, Unit; VB₁₂, Vitamin B₁₂.

I. INTRODUCTION

Helicobacter pylori are a member of the major agents responsible for digestive track disorders, like peptic ulcer diseases, GC, gastritis, and MALT. Helicobacter pylori are thought to be spread from one person to another via the oral-to-oral, gastro-to-oral, and fecal-to-oral transmitting route (Kusters et al., 2006); these bacteria can survive in an acidic environment because of their virulence factors, which include; the outer inflammatory protein A, the vacuolating cytotoxin A gene, the cytotoxin associated gene A, the duodenal ulcer promoting gene A, and surface antigens (Ag), which can cause chronic gastric antrum inflammation (Yamaoka et al., 1999). Furthermore, it has been isolated from extra-gastrointestinal materials like; blood, atherosclerotic plaques, or dental plaques, indicating the possibility of extra-gastrointestinal invasion (Figura et al., 2010).

Various invasive and non-invasive identification assays are available for H. pylori detection. Tests using stomach biopsies, such as rapid urease test, histopathological examination, culture, and molecular tests, are examples of invasive techniques, whereas non-invasive tests involve; SAT, urea breath test, and serology (Nair et al., 2018). Helicobacter pylori may be detected by endoscopic examination, and the endoscopic imaging can reveal the histological features of the stomach mucosa with a diffused redness of the stomach lumen and patchy fundal hemorrhage being strong indicators of H. pylori positivity (Chatrangsun and Vilachone, 2021).

Two types are available for SAT; enzyme-linked immune-sorbent assay (ELISA) and immunochromatography assay. Immunochromatography test is more popular today due to their affordability, simplicity of use, and quickness. The SAT accuracy may vary depending on the population due to variations in the epitopes of H. pylori isolates. It was confirmed in a previous study that SAT can be used as a reliable substitute for invasive tests to diagnose H. pylori bacteria (Nair et al., 2018).

Helicobacter pylori-infected patients develop both localized and systematic immune defenses, and rapid serological testing can quickly identify specific H. pylori
Abs, which are widely available in most hospitals or clinical laboratories as non-invasive techniques, that are rapid, easy, cheap, don’t require any particular equipment or skill and can be used to test patients immediately for *H. pylori* Abs after routine surgery. The most popular serologic test is ELISA since it effectively screens huge populations (Yu et al., 2022).

Autoimmune gastritis and *H. pylori* gastritis are typically considered distinct conditions, yet they may be correlated. Some people get AIG after *H. pylori* infection because the H⁺/K⁺-adenosine triphosphatase (ATPase) proton pump on the stomach parietal cells and *H. pylori* Ags are homologous; thus, T-cell dependent immunological defense against the *H. pylori* can thereby trigger autoimmune damage of the oxyntic gastric mucosal lumen (Choudhuri et al., 2021). One of the distinguishing characteristics of AIG is the loss of parietal cells, which secrete hydrochloric acid (HCl) and intrinsic factors (IF). Autoimmune gastritis cause remains controversial. It could be due to infection with *H. pylori* or stomach autoimmunity. During the initial stages of the AIG, PCA specific for H⁺/K⁺-ATPase Ags are recorded with higher percent, and during the AIG chronic phase, immunological reaction due to PCA and/or IF auto-Abs causes mucosal atrophy, which finally results in the complete loss of parietal cells. Micronutrients like VB₁₂ and iron may not be well absorbed if insufficient stomach acid and IF secretion is insufficient. Autoimmune gastritis leads to the development of pernicious anemia (PA), which is defined by decreasing VB₁₂ absorption and megaloblastic anemia. Patients with PA may exhibit iron insufficiency and/or iron deficiency anemia (IDA) because of achlorhydria, reduced iron absorption (Demir et al., 2020). Current research is designed to detect the involvement of *H. pylori* bacteria in the induction of AIG and to assess the sera levels of VB₁₂ and iron between individuals with and without *H. pylori* disease, and to determine the influence of AIG on levels of the two former biomarkers.

II. MATERIALS AND METHODS

A. Study Design/Subjects:

A control case research was conducted in Thi-Qar Governorate, Iraq, in November-2021 and June-2022. The study was included 55 subjects (27 males and 28 females) with *H. pylori*-associated diseases and 35 healthy individuals (19 females and 16 males), which regarded as a healthy control (HC). The eligibility criteria of both study groups mentioned below were followed stringently. The age of *H. pylori*-associated patients (HPP) ranged from 13 to 75 years, whereas the HC age was 11 years old to 70 years old. The participants were recruited from the Endoscopy Units of Imam Al-Hussein and Al-Nasiriyyah Teaching hospitals.

B. Ethics approval/consent to participate:

Each participant in the current research was informed of written consent to full-fulf the international research ethics consideration criterion, and a recent article was authorized by the committee of ethical consideration of the Health Department of Thi-Qar, Ministry of Health/Environment, Iraq (No: 1254).

C. Study groups selection criteria:

For HPP, patients who met any one of the subsequent criteria were omitted; under antibiotics drugs for the last 14 days, taking non-steroidal anti-inflammatory medications during the last week, receiving corticosteroid drugs during the last month, receiving blood transfusions in time of the last six months, had recent surgery in the time of the last six months, the existence of any autoimmune or chronic diseases, and use of any biological agents. Patients were classified as HPP if they met the following criteria; clinical findings were associated with *H. pylori* disease, endoscopic observations that were congruent with *H. pylori* infection, SAT positive for *H. pylori* by *H. pylori* Ags rapid test, positive serology for *H. pylori* by *H. pylori* IgG-ELISA technique and *H. pylori*-Abs cassette assay, and without any of the eligibility mentioned above guidelines (exclusion criteria). The HC subject's inclusion guidelines were as follows; had none of those as mentioned earlier HPP group exclusions criteria, no *H. pylori* infection history or its associated diseases history, body mass index, and age were matched with HPP, in addition, individuals with a mild infection were excluded.

D. Endoscopy:

Endoscopic examination was performed at Al-Nasiriyyah and Imam Al-Hussein Teaching Hospitals Endoscopy Units. Before starting the endoscopic examination, the patients fasted for at least eight hours, then under local pharyngeal anesthetic, a physician performed a diagnostic esophagus-gastro-duodenoscopy using Olympus Endoscopy (Japan) for all HPP for their primary endoscopic findings.

E. Samples collection:

For the HPP group, in a sterile screw-cap vial, a fresh fecal sample of about two grams (g) for solid and/or semisolid fecal and 2 milliliters (ml) for liquid feces was obtained, and in the site was used for SAT. Then from each subject of both study groups, 5 ml of venous blood was drawn using disposable syringes (Medeco, Belgium) and a gel tube (China). To obtain serum, at the end of the clotting process at room temperature, the blood specimens were spun at 3,000 cycles per minute for ten minutes using a centrifuge (Hettich, Germany). Until their needed for serological assays, each serum specimen was separated into multiple portions and kept in Eppendorf tubes within 80 °C.

F. Fecal antigen test:

Detecting *H. pylori* Ags in human feces samples using a lateral flow immune-chromatographic assay. Kit for the *H. pylori*-Ag cassette (*Linear, Spain, Reference: 4245122*) was used to perform this test on the sample collection site.

G. Serum anti-Helicobacter pylori Abs (cassette assay):
The *H. pylori* Abs cassette assay is a rapid visual qualitative immune assay for the presumptive identification of certain serum anti- *H. pylori* Abs (Linear, Spain, Ref: 4260240). This assay was performed at the Teaching Hospital of Imam Al-Hussein, Health Office of Thi-Qar.

**H. Serum anti-Helicobacter pylori Ab (IgG) assay:**

Anti- *H. pylori* IgG-Abs was detected and titrated in a unit (U)/ml via *H. pylori*-IgG-ELISA kit (Demeditec, Germany, Reference: DEHEL01) that depended on the method of ELISA technology and performed at the Teaching Hospital of Imam Al-Hussein, Health Office of Thi-Qar. The results of this assay were regarded as *H. pylori*-positive when the level of anti- *H. pylori*-IgG Abs were more than 12 U/ml.

**I. Determination of parietal cells auto-Abs level:**

At the Teaching Hospital of Imam Al-Hussein, Health Office of Thi-Qar, the assay protocol of the human anti-parietal cells Abs ELISA ( *Shanghai YL Biont, China*, Catalog No: YLA4183HU) was conducted. Using this kit based on biotin double Abs sandwich technology, human PCA was detected and titrated in serum. The findings have been recorded in picograms (pg) per ml.

**J. Determination of vitamin B\textsubscript{12} concentration:**

This test was performed at the Teaching Hospital of Imam Al-Hussein, Thi-Qar Health Department. The detection and titration of human VB\textsubscript{12} in serum were performed using an ELISA kit for the human VB\textsubscript{12} ( *Shanghai YL Biont, China*, Catalog No. YLA0810HU), and the results were expressed in picomoles (pmol)/liter (L).

**K. Determination of serum iron concentration:**

This test was conducted at the Teaching Hospital of Imam Al-Hussein, Health Office of Thi-Qar, in which human iron was measured in serum using a direct iron method (Ferene) kit (Biolabo, France, Ref: 92108). Ascorbic acid converts iron (Fe\textsuperscript{3+}) into iron (Fe\textsuperscript{2+}) after dissociating iron-transferrin bound in an acid media. Then, iron (Fe\textsuperscript{2+}) and 3-(2-Pyridyl)-5-,6-difuryl-1-,2-,4-triazine-disulfonate (Ferene) combined to form a colorful complex. The concentration of iron in the initial specimen was proportional to the colored complex absorbance. The findings have been recorded in micrograms (µg)/deciliter (dl).

**L. Statistical analysis:**

Microsoft Office Excel 2010 program and statistical software for social sciences (version 27) were used for the statistical analysis. The frequency, percentage, and mean were used to express the data. Chi-Square statistical test, simple linear regression, and Pearson’s correlation coefficients were used to calculate the correlations between biomarkers. At a p-value of lower than 0.05, the statistics were regarded as statistically significant.

**III. RESULTS**

Ninety participants were included in the recent research after strictly applying the inclusion and exclusion criteria previously stated in the materials and methods. Out of 90 participants, 55 (27 males and 28 females) had clinical signs of *H. pylori*-associated digestive tract disorders with ages ranging from 13 to 75 years, which were classified as the HPP group. The remaining 35 individuals (16 men and 19 women) were considered the HC group. The HC subject’s age range was 11 to 70 years.

Parietal cell auto-Abs results in all research groups are illustrated in Figure (1). The frequency % of serum PCA positivity was significantly (p<0.05) elevated in the HPP group (18.2%) in comparison with the HC subjects (11.4%). The mean titer of serum PCA was higher (45.3 pg/ml) in the HPP compared with the HC subjects (39.1 pg/ml). Moreover, the differences in mean titer among both cohorts were statistically valuable (P:<0.05).

**Figure (1): Serum parietal cells autoantibody results (PCA, parietal cells autoantibody, pg/ml, picograms/millilitre; HC, healthy control; HPP, Helicobacter pylori-associated patients; Positive, >60 pg/ml, Negative, ≤60 pg/ml).**

Figure (2) shows the results of the VB\textsubscript{12} in both research groups. The vast majority of HPP group subjects had the highest frequency percent of below-normal level serum VB\textsubscript{12} (83.6%) compared to the subjects of the HC group (0%) with statistical differences (p<0.05). The mean titer of this biomarker were statistically (P<0.05) lower within the HPP group (102 pmol/l) than in the HC group (337 pmol/ml).
Figure (2): Serum vitamin B12 results (VB12, vitamin B12; pmol/l, picomoles/liter; HC, healthy control; HPP, Helicobacter pylori associated patients; Below-normal, <148 pmol/l; Normal, 148-185 pmol/l; Above normal, >185 pmol/l).

Figure (3) shows the serum iron values among both research groups. In the HPP, the % of iron below normal concentration was statistically (P<0.05) increased (50.9%) compared to the HC subjects (5.7 percent), and there was the lowest mean value (64.9 µg/dl) in comparison to the HC group (87 µg/dl). Between both study groups, the mean titer differed significantly (p<0.05).

![Figure 3: Serum iron results (µg/dl)](image)

Table (1) illustrates the association between PCA and anti- H. pylori (IgG) Abs in all research subjects. The results showed that the HPP with highly positive levels of anti- H. pylori (IgG) Abs had statistically (p<0.05) higher PCA positivity 10/52 (19.2%) than HPP with weak positive anti- H. pylori-IgG Abs 0/3 (0%). For HPP with higher positivity of IgG anti- H. pylori-Abs, the mean titers of PCA were statistically (P<0.05) higher (458 pg/ml) in comparison with low positive HPP (23.83 pg/ml). The regression analysis in Figure (4) demonstrated a statistically valuable (p<0.05) positive association among sera anti- H. pylori-IgG Abs and PCA levels in both research cohorts.

**TABLE (1):** Correlation between parietal cells autoantibodies and anti-Helicobacter pylori (IgG) antibodies

<table>
<thead>
<tr>
<th>Markers</th>
<th>Positive (pH)</th>
<th>Negative (pH)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB12 mean (pmol/l)</td>
<td>337</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron mean (µg/dl)</td>
<td>87</td>
<td>64.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The correlation among VB12 and IgG anti- H. pylori Abs was illustrated in Table 2. For HPP with highly positive anti- H. pylori-IgG Abs levels, the % of serum VB12 below-normal levels was highest 44/52 (84.6%) than in HPP with low positive anti- H. pylori (IgG) Abs levels 2/3 (66.7%). Regarded to VB12 concentration, the differences between patients with high and low positive IgG anti- H. pylori Abs levels were statistically valuable (p<0.05). For means values, the findings showed that the VB12 mean value was lower statistically (p<0.05) in HPP with a highly positive anti- H. pylori-IgG Abs concentration (102 pmol/l) than low positive one (131.3 pmol/l). The linear analysis of regression (Figure 5) illustrated a noticeable (p<0.05) inverse association among IgG anti- H: pylori Abs and VB12 in both research cohorts.

**TABLE (2):** Relationship among anti-Helicobacter pylori (IgG) antibody and vitamin B12

<table>
<thead>
<tr>
<th>Markers</th>
<th>Below N (&lt;148)</th>
<th>Above N (≥148)</th>
<th>N (148-185)</th>
<th>Above N (≥185)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB12 mean (pmol/l)</td>
<td>337</td>
<td>102</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VB12 mean (pmol/l)</td>
<td>87</td>
<td>64.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure (4):** Regression analysis between (IgG) Abs against Helicobacter pylori and parietal cells autoantibody (HPP, Helicobacter pylori-associated patients; HC, healthy control; PCA, parietal cells autoantibody; IgG, immunoglobulin gamma; pg/ml, picograms/milliliter; U/ml, units per milliliter; Hp, Helicobacter pylori; L, low (13 to 33 U/ml); H, high (>33 U/ml).

![Figure 4: Regression analysis](image)

**Figure (5):** R analysis of regression (Figure 5) illustrated a noticeable (p<0.05) inverse association among IgG anti- H: pylori Abs and VB12 in both research cohorts.
The correlation of serum iron and serum IgG anti-\textit{H. pylori} Abs was illustrated in Table (3). A significantly ($p<0.05$) lower frequency percent of normal serum iron levels 22/52 (42.3\%) was observed in HPP with a highly positive concentration of anti-\textit{H. pylori}-IgG Abs compared to HPP with weak positive anti-\textit{H. pylori} (IgG) Abs concentration 3/3(100\%). For mean value, the findings showed that the mean value of iron was low in the HPP with higher positive IgG anti-\textit{H. pylori} Abs concentration (63.5 µg/dl) and higher among low positive IgG anti-\textit{H. pylori} Abs level (89.3 µg/dl) with a statistical difference ($p<0.05$). The HPP showed a significant negative association ($p<0.05$) among IgG anti-\textit{H. pylori} Abs and iron sera levels, while the HC subjects showed a non-significant positive correlation ($p>0.05$) as illustrated in Figure 6’s regression analysis.

**TABLE (3): Correlation between anti-\textit{Helicobacter pylori} (IgG) antibody and iron**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Below N ($\leq$60) Mean</th>
<th>Normal (60-179) Mean</th>
<th>Above N ($\geq$180) Mean</th>
<th>Total Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPP (pg/ml)</td>
<td>28.53(32) 18.5 224/52 82.9 22/59 200 52/100</td>
<td>35(100)</td>
<td>55(100)</td>
<td>102</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HC (pg/ml)</td>
<td>28/50(30) 18.5 254/50 81.6 23/59 200 53/100 84.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=31)</td>
<td>28/5.7 26 30/49.9 88.8 26/0 25/100</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n=45)</td>
<td>28/57.5 36 30/49.9 88.8 26/0 25/100</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HPP, \textit{Helicobacter pylori}-associated patients; HC, healthy control; FR, frequency; (\%), percent; \textit{Hp}, \textit{Helicobacter pylori}, IgG, immunoglobulin gamma; \textit{n}, number; U/ml, units/milliliter; µg/dl, micrograms/deciliter; N, normal; L, low (13 to 33 U/ml); H, high (53 U/ml).

**Figure (5): Regression analysis between (IgG) Abs against \textit{Helicobacter pylori} and vitamin B$_{12}$ (HPP, \textit{Helicobacter pylori}-associated patients; HC, healthy control; IgG, immunoglobulin gamma; pmol/l, picomoles/liter; U/ml, units/milliliter).**

Table (4) revealed the association between the PCA and VB$_{12}$ in all research cohorts. The findings illustrated non-statistical significance ($p>0.05$) in the correlation among the PCA positive and negative subjects with frequency \% of VB$_{12}$ level among both study groups. The same was true for VB$_{12}$ mean titer. For regression analysis (Figure 7), the HPP group showed no statistical difference ($p>0.05$) and a weak inverse association among PCA and VB$_{12}$ levels, whereas, in the HC group, there was a positive association between the former biomarkers with no statistical different ($p>0.05$).

**TABLE (4): Relationship among parietal cells autoantibodies and vitamin B$_{12}$**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Below N ($\leq$60) Mean</th>
<th>Normal (60-179) Mean</th>
<th>Above N ($\geq$180) Mean</th>
<th>Total Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n=45)</td>
<td>22(4.4) 20(4.4) 79.3 20(4.4) 200</td>
<td>83.6</td>
<td>59.1</td>
<td>102.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Positive (n=55)</td>
<td>0(0) 0 0 0 31/100</td>
<td>52.7</td>
<td>51/100</td>
<td>103.5</td>
<td></td>
</tr>
<tr>
<td>Total (n=55)</td>
<td>64.9</td>
<td>83.6</td>
<td>59.1</td>
<td>102.5</td>
<td></td>
</tr>
</tbody>
</table>

HPP, \textit{Helicobacter pylori}-associated patients; HC, healthy control; PCA, parietal cells autoantibodies; pmol/l, picomoles/liter; FR, frequency; (\%), percent; pg/ml, picograms/milliliter; \textit{n}, number; N, normal; Positive, >50 pg/ml; Negative, <50 pg/ml.

**Figure (6): Regression analysis between (IgG) Abs against \textit{Helicobacter pylori} and iron (HPP, \textit{Helicobacter pylori}-associated patients; HC, healthy control; IgG, immunoglobulin gamma; U/ml, units per milliliter; µg/dl, micrograms/deciliter).**

**Figure (7): Regression analysis of parietal cells autoantibody and vitamin B$_{12}$ (HPP, \textit{Helicobacter pylori}-associated patients; HC, healthy control; PCA, parietal cells autoantibodies; pmol/l, picomoles/liter; pg/ml, picograms/milliliter).**

Table (5) illustrates the relationship between serum PCA and serum iron levels. The differences in the frequency \% of iron levels between subjects with positive and negative serum PCA were insignificant ($p>0.05$) for both study groups. The same results profile was documented with a mean iron titer, as the Table shows. The findings of the current research (Figure 8) showed a simple opposite relationship ($p>0.05$) among PCA and iron sera concentrations in HPP, whereas the observations of the HC subjects showed a slight non-significant positive relationship ($p>0.05$).

**TABLE (5): Relationship among parietal cells autoantibodies and iron**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Below N ($\leq$60) Mean</th>
<th>Normal (60-179) Mean</th>
<th>Above N ($\geq$180) Mean</th>
<th>Total Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n=45)</td>
<td>50(100) 30(60) 40(80) 100(100)</td>
<td>50(100)</td>
<td>50(100)</td>
<td>100</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Positive (n=55)</td>
<td>0(0) 0 0 0 51/100</td>
<td>97.5</td>
<td>51/100</td>
<td>102.5</td>
<td></td>
</tr>
<tr>
<td>Total (n=55)</td>
<td>55(100) 30(60) 40(80) 100(100)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Figure (8):** Relationship among parietal cells autoantibodies and iron.
Figure (8): Regression analysis of parietal cells autoantibody and iron (HPP, *Helicobacter pylori*-associated patients; HC, healthy control; PCA, parietal cells autoantibodies; µg/dl, microgram per deciliter; pg/ml, picogram per milliliter).

The relationship between VB₁₂ and iron sera concentrations in all study groups is listed in Table (6). For HPP, there were no valuable differences (p > 0.05) in the frequency % as well as the mean titer of iron between subjects with below-normal, normal, or above-normal VB₁₂ levels. In both study groups, data in Figure (9) indicated a poor, non-significant (p > 0.05) negative relationship between the VB₁₂ and iron levels.

**TABLE (6): Correlation between vitamin B₁₂ and iron**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Below N (n=50)</th>
<th>Normal (n=56)</th>
<th>Above N (n=16)</th>
<th>Total n=102</th>
<th>Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPP (µg/dl)</td>
<td>20.0 (5.0)</td>
<td>35.0 (6.0)</td>
<td>100 (20.0)</td>
<td>155.0 (30.0)</td>
<td>90.0</td>
<td>0.000</td>
</tr>
<tr>
<td>HC (µg/dl)</td>
<td>25.0 (5.0)</td>
<td>35.0 (6.0)</td>
<td>100 (20.0)</td>
<td>155.0 (30.0)</td>
<td>90.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Figure (9): Analysis of regression between vitamin B₁₂ and iron (HPP, *Helicobacter pylori*-associated patients; HC, healthy control; µg/dl, microgram per deciliter; pmol/l, picomole per liter).**

**IV. DISCUSSION**

*Helicobacter pylori* is a flagellated, micro-aerophilic, gram-negative bacteria that preferentially invades the gastrointestinal mucosal tissues and is regarded as one of the commonest prevalent infectious agents in the world which 50% of people worldwide are afflicted with it (Huang et al., 2016). The hallmark of the AIG is the loss of stomach parietal cells, resulting in the lack of IF and a decrease in acid output capacity. These modifications cause malabsorption of various micronutrients, including iron, VB₁₂ (PA), as well as other micronutrients. Since many previous studies have been concerned with PA alone for several years, there is confusion between the two conditions (AIG and PA). The key auto-Ags that autoreactive T lymphocytes target in AIG is the stomach proton pump-H⁺/K⁺ ATPase. Parietal cells auto-Abs are the serological biomarker of AIG secreted by the active B-cells in the presence of T-cells. It is still unclear whether *H. pylori* infection favors and/or activates the autoimmune process (Lenti et al., 2020). Consistent with these observations, current study outcomes (Figure 1) showed that the PCA positivity was significantly (p < 0.05) elevated in the HPP in comparison with the HC subjects. Serum PCA is frequent in adult individuals with *H. pylori*-associated disease, and the degree of stomach body inflammation and mucosal loss is correlated with the presence of PCA, especially those reactive with canalicular structures within parietal cells (Faller et al., 1997). Lewis blood group Ags presented on gastric mucosa epithelial cells have been hypothesized to have a molecular similarity with *H. pylori* Ags that contributes to the generation of PCA (Negrini et al., 2020).

According to the current study, HPP had significantly lower VB₁₂ levels than HC subjects (Figure 2). Overall, current findings aligned with another research (Carmel et al., 2001), which had shown that *H. pylori* infection was related to VB₁₂ deficiency. In the communities where *H. pylori* bacteria is more prevalent, clinical repercussions due to VB₁₂ insufficiency are likely to occur more frequently. Due to *H. pylori* invasion of the stomach mucosal tissues is associated with clinical and histopathological evidence of long-standing gastritis that is accompanied by localized and systematic immune responses, the former study found that a higher percentage (64%) of VB₁₂ insufficiency in patients with *H. pylori*-associated disease. Following antibiotics treatment and elimination of the *H. pylori* bacteria, normal gastric epithelial appearance, remission of gastritis, and the mucosal immune defense against *H. pylori* are restored (Kadhim et al., 2018). The most common etiology of megaloblastic anemia is VB₁₂ deficiency, which can also result in neurological issues (Kadhim et al., 2018). Another explanation for current study findings (Figure 2) of VB₁₂ insufficiency in the HPP group; which is correlated with poor food intake, illium disorders that cause impaired absorption, problems involving the release of gastric pepsin, and IF secretion by parietal cells (Ravi et al., 2017).

In the current investigations, the serum iron in the HPP group was significantly decreased compared with HC subjects (Figure 3). This result agreed with previous
studies' findings (Zakaria and Ahmed, 2009). According to the current findings, *H. pylori* bacteria reduced iron absorption. This issue could be due to; direct competition for available iron with the patients or obstructing iron absorption. Understanding the details of the correlation between anemia and *H. pylori* bacteria often requires taking into account many potential pathways like irregular bleeding. Another intriguing possibility is that *H. pylori* could work as a parasitic relationship that battles the host for iron in vivo, acting as an iron-acquisition mechanism. *Helicobacter pylori* colonization can manifest in diffuse corpus gastritis, and the presence of this condition may significantly contribute to stomach hypocaidoci (Queq et al., 2011) and thus leads toIDA.

The findings of the current research in Table (1) and Figure (4) demonstrated a statistically significant positive correlation among anti-*H. pylori* (IgG) Abs and PCA positivity in the HPP group. In matching with recent study findings, another previous research showed that the seropositive individuals for *H. pylori* had a high occurrence of PCA (Šterzl et al., 2008). Results of the current study suggest a potential correlation between PCA positivity and the severity of infection with *H. pylori* (as higher anti-*H. pylori*-IgG Abs confirmed it) in which the occurrence of PCA positivity was higher among HPP with increased anti-*H. pylori* (IgG) Abs. Previous study findings speculated that serum anti-*H. pylori* (IgG) Abs mean titer was positively related to bacterial density in the stomach mucosa and the grade of histopathological gastritis (Tu et al., 2014), and the *H. pylori* density was negatively correlated with VB12 serum concentration (Serin et al., 2002). Following these observations, the recent research findings (Table 2 and Figure 5) showed a significant negative relationship between VB12 and IgG anti-*H. pylori* Abs levels among HPP. A potential cause of IDA that does not respond to iron therapy is *H. pylori* gastritis (Jasem et al., 2011). Current study findings verified the theory that infection with *H. pylori* was linked with lower iron concentrations (Figure 3) and the mean titer of IgG anti-*H. pylori* Abs were inversely linked with blood iron levels in HPP (Table 3 and Figure 6). This theory was reported by several previous types of research with various explanations of the pathways whereby *H. pylori* bacteria hampered iron absorption. Baysoy et al. (2004) noted a relationship between *H. pylori* infection and lower serum iron levels, as well as a depletion in the ascorbic acid level in gastric secretion. Capruso et al. (2001) showed that IDA participants infected by *H. pylori* had greater intra-gastric pH and gastrin serum levels. They supported the hypothesis that *H. pylori* bacteria might be the main cause of AIG, which results in achlorhydra and stomach low acidity levels, and the current paper reported this finding. In Alaska, where *H. pylori* bacteria is highly prevalent, a study involving 2080 adult patients recorded a statistical correlation among anti-*H. pylori* (IgG) Abs positivity and lower ferritin concentration (Yip et al., 1997). Current study findings speculated that serum iron level was reduced in HPP that had increased IgG Abs against *H. pylori*, and the infection with this bacteria affects iron metabolism in humans.

Previous findings demonstrated that *H. pylori* infection causes stomach autoimmunity (Presotto et al., 2003).

Regarded to the association between VB12 level and AIG, conflicting findings have been recorded in many previous types of research. Although gastric atrophy and PCA were more common in individuals with low serum VB12 levels, this was statistically non-significant; nevertheless, the incidence of anti-IF auto-Abs was statistically significant in those subjects (Ayesh et al., 2013). The present study showed non-significant differences in VB12 levels between PCA-positive and PCA-negative subjects among all study groups (Table 4 and Figure 7). These conflicting results may be due to differences in patient selection criteria and in the methods of the measurement of the biomarkers as well as it has been reported that oral VB12 supplementation is a secure and successful treatment for the VB12 deficient state. Oral therapy with VB12 is beneficial even when IF is absent to facilitate the intestinal uptake of VB12 (PA) or in conditions that alter the typical uptake area in the terminal ileum (Oh and Brown, 2003). Therefore, even for individuals who exhibit PCA in their sera, PA in this particular subset of PCA-positive subjects may be avoided with adequate dietary intake of VB12. Also, PCA-positive subjects need sufficient time to develop the AIG, ultimately leading to PA (Lahner and Annibale, 2009). Therefore, it is possible that subjects with low PCA serum mean titer or those with moderate or high PCA titers of shorter duration of onset may not have PA during the period of samples taken (in the current article). Recent research reported no statistical difference in iron levels among subjects with or without PCA positivity in both study groups (Table 5 and Figure 8). Consistent with these findings, another previous study reported that PA was present in just 13% of PCA-positive patients (Sun et al., 2013). According to the current research findings, PCA positivity was not the only issue contributing to iron insufficiency. Other causes that may contribute to iron insufficiency include; inadequate iron intake, drug-induced or malabsorption of iron, bacterial overgrowth, tapeworm infestation, and improper iron transport (Oh and Brown, 2003). Parietal cells auto-Abs are often not specific; it is frequently detected in people with autoimmune endocrine diseases and is also detectable in 3% to 10% of normal individuals (as in present study findings: 11.4%, Figure 1) (Sun et al., 2013). Thus despite being PCA-positive, a particular group of individuals may not have an iron insufficiency. The recent findings revealed non-significant differences in iron levels among subjects with below-normal, normal, or above-normal VB12 levels in the HPP group (Table 6). In contrast, the findings within Figure 9 showed an inverse non-significant correlation between both biomarkers (Iron x VB12) in both study groups—conflicting findings from different previous studies on VB12 level after IDA treatment. Roberts et al. (1971) and Akhmeteli et al. (2005) recorded that VB12 concentrations were unaltered in IDA subjects receiving iron supplements, consistent with current paper findings, Remacha et al. (2015) reported that pharmacological iron therapy raised VB12 levels. Acik and Ayyun (2020) showed that sera concentrations of VB12 and folate were decreased in response to iron treatment. *Helicobacter pylori* infection is incredibly common in Iraq. Therefore, the limited sample size of the HC group
was one of the limitations of this study. This issue may cause the absence of statistically significant differences between HPP and controls. Some of the recent study subjects may have taken some medications (as supplements), which were not recorded in the patient record or on the patient claims, and this could have an impact on the concentration of the current study biomarkers in their sera. Other limitations were the specificity and sensitivity of the diagnostic kits of the biomarkers, which may not reflect the actual concentration of the original samples. The correlation between PCA, VB$_{12}$, and iron biomarkers with the degree and severity of histological findings and the presence of peptic ulcer disorders or stomach cancer are needed in future studies.

V. CONCLUSIONS

The results indicated that PCA positivity, low VB$_{12}$ level, and low iron level were associated with infection by *H. pylori*. For subjects infected by *H. pylori*, routine testing for PCA, VB$_{12}$, and iron is required. Anti-*H. pylori* (IgG) Abs sera concentrations are a reliable biomarker for developing AIG and lower VB$_{12}$ and iron levels. It is recommended to treat people with high IgG anti-*H. pylori* Abs titers for *H. pylori* screened prophylactic therapy for AIG and iron and VB$_{12}$ deficiencies. Parietal cells auto-Abs were not associated with VB$_{12}$ and iron levels, and the two former biomarkers were not correlated. Different age-matched large-scale prospective investigations are needed to establish a clear correlation between PCA, VB$_{12}$, and iron in people with *H. pylori*-associated diseases.

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