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# Correlation between *Helicobacter pylori* Infection and The Level of Serum Inflammatory Markers

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## Abstract

**Background** *Helicobacter pylori* (*H. pylori*), is a Gram-negative bacterium that colonizes the human stomach. Persistence of *H. pylori* infection may lead to various gastrointestinal pathologies such as gastric cancer.

**Objective** To investigate the correlation between *H. pylori* antigen in stool and antibody with serum interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α).

**Methods** The participants were assigned into patients and controls, for both groups, rapid urease test, *H. pylori* stool antigen test, Anti-*H. pylori* IgA, and IgG, serum Il-6, and serum TNF-α were measured.

**Results** The mean serum levels of the IgA, IgG, IL-6, and TNF-α for the patients were as follows: 32.56±0.51 U/ml, 18.37±0.39 U/ml, 71.05±1.09 pg/ml, 54.72±1.01 pg/ml, respectively, while those of the controls were: 7.97±0.35 U/ml, 2.09±0.21 U/ml, 11.69±0.50 pg/ml, 3.76±0.23 pg/ml, respectively. The comparisons of all four markers showed very highly statistically significant difference between patients and controls (P <0.001). The mean serum concentrations of the IgG showed a highly statistically significant difference (P <0.001), while all other comparisons indicated no statistically significant difference (P = 0.76, P = 0.76, P = 0.70) for the IgA, IL-6, and TNF-α comparisons, respectively.

**Conclusion** Infection with *H. pylori* can significantly alter the diagnostic markers and the markers commonly used to determine post-infection inflammatory response thus those markers can be utilized to diagnose and/or follow up infection progress.

**Keywords** H. pylori, IgA, IgG, IL-6, TNF-α

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**List of abbreviations:** ELISA = Enzyme-linked immunosorbent assay, *H. pylori* = *Helicobacter pylori*, IL = Interleukin, IgA = Immunoglobulin A, IgG = Immunoglobulin G), TNF-α = Tumor necrosis factor-alpha

## Introduction

*Helicobacter pylori* (*H. pylori*) infection, near or about half of the world population were infected with it, which is remain a consider as public health problem. Early detection and treatment of *H. pylori* infection is essential to stop the infection from spreading because it can cause inflammation, and this led to different disease such as stomach ulcers, and cancers, among other gastric diseases [1]. In highly industrialized nations, the prevalence of *H. pylori* infection has been falling, but in developing and recently industrialized nations, the prevalence has stabilized at a high level [2,3].

Different types of immune cells have been shown to generate both pro-inflammatory and anti-inflammatory cytokines in response to the *H. pylori* infection in the stomach mucosa. Numerous of these cytokines are released into

the bloodstream both at the infection site and generally [4]. A variety of human cells, including monocytes, lymphocytes, macrophages, endothelium and intestinal epithelial cells, express the multifunctional, pleiotropic, and multipotent cytokine interleukin-6 (IL-6), whose gene is found on chromosome 7. It serves as a crucial modulator of inflammation and immunity [5].

The immune system reacts to invasive infections mostly through the production of pro-inflammatory cytokines such tumor necrosis factor-alpha (TNF-α) [6]. TNF-α, an endotoxin-induced glycoprotein, belongs to the family of cytokines that are involved in acute phase response and systemic inflammation. TNF-α is primarily involved in immune cell regulation. This cytokine can cause fever, inflammation, apoptosis, and cachexia since it is an endogenous pyrogen [7]. When compared to non-infected individuals, those with *H. pylori* infection had higher levels of TNF-α [8].

Various approaches are utilized to achieve a de novo diagnosis or to monitor the disease associated with *H. pylori* infection.

Traditionally, diagnosis has been accomplished using a mixture of invasive and noninvasive tests. Given the extensive range of diagnostic procedures available, tests with high diagnostic accuracy should be utilized in clinical practice under specified conditions, and their sensitivity and specificity are currently assumed to be Greater than 90%. The method to be chosen is based on the clinical circumstances of the patient to be examined, the availability of the test, the probability ratio of positive and negative results, and its cost-effectiveness [9].

This study aimed to search for the relation of serum inflammatory markers and *H. pylori* infection.

## Methods

### Patients

Eighty participants (45 females and 35 males), aged 19 to 71 years, were selected for this study. The participants were referred to the Gastroenterology Department at Baghdad Medical City between January 5 and April 10, 2024. All individuals reported recurrent gastrointestinal pain and symptoms such as intermittent colic, nausea, and vomiting. Exclusion criteria included prior use of anti-*H. pylori* medications. Ethical approval for the study was obtained from the hospital's ethics committee. The participants were divided into two groups: 50 patients and 30 controls who were persons non infected with *H. Pylori*.

### *H. Pylori* Antigen Rapid Test (stool sample)

Human *H. pylori* Rapid Test (Cassette) Antigen Rapi Card Insta Test (Cat. No. 118564-1-44 Cortez diagnostics, USA) was used as per manufacturer instruction for the determination of the presence of *H. pylori* antigen in the stool of candidates. The result is positive if both the C line and T line appear, indicating that the IgG antibodies specific to *H. pylori* are detected. If only the C line appears in the control region, the test indicates that no antibodies to *H. pylori* are detected and the result is negative.

### Serological test (blood sample)

One step *H. pylori* / Serum / WB Rapi Card™ Insta Test (Cat. no. 118562-19-25 CTK Biotech, USA) according to the instructions of the manufacturer. The test can be interpreted as follow: Positive: If both the C line and T line appear, the result indicates that the antigen of *H. pylori* are detected and the result is positive. A faint line in test region indicates a borderline specimen, which should be re-tested using an alternative method for confirmation. Negative: If only the C line appears in the control region, the test indicates that no antigen of *H. pylori* is detected and the result is negative. Invalid: When no control line appears within 5 min, repeat the test with a new test device.

### Enzyme-linked immunosorbent assay (ELISA) method (blood samples)

Serum samples were utilized for ELISA method for the quantitation of anti-*H. Pylori* IgA and IgG antibodies. ELISA kits used for this purpose were Human *H. pylori* IgA ELISA Kit (Catalogue No.

PT_H.P. IgA_96_02 PISHTAZTEB DIAGNOSTICS. Germany) and Human *H. pylori* IgG ELISA kit (Catalogue No. PT-H.P. IgG-96-02 PISHTAZ TEB DIAGNOSTIC. Germany). The test steps were applied by strictly following the rules of the manufacturing company. For the determination of serum levels of IL-6, Human IL-6 ELISA kit was used (No. EH2IL6, Invitrogen, Austria) and Human TNF-α Instant ELISA Kit (Cat. No. BMS223INST, Invitrogen, Austria) was used for the assessment of TNF-α serum levels.

**Statistical analysis**

The statistical package for social sciences (SPSS Inc., Chicago, IL, USA), version 20 used for statistical analysis. Categorical data formulated as count and percentage. Numerical data were described as mean and standard deviation. Independent unpaired t-test used for comparison between two groups while analysis of variance (one-way ANOVA) was used to compare between more than two groups. The lower level of accepted statistical significant difference is equal or less than 0.05.

## Results

The results of this study indicated that both male patients and female patients constituted 31% for each, whereas control males and control females who tested negative represented 21% and 16%, respectively (Figure 1).

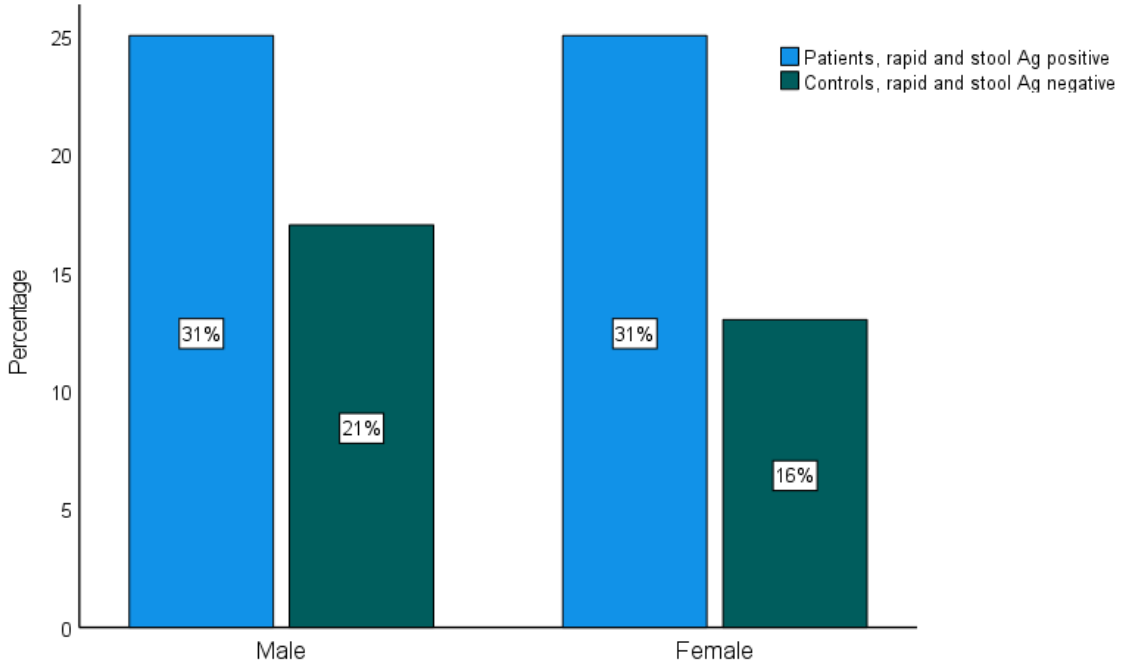


**Figure 1. Gender distribution across patients and controls groups**

Table (1) shows the mean age differences between patients and controls, it is revealed that the mean age of the patients was 44.49±1.61 years while that of the controls was 48.39±1.49 years; there was no statistically significant difference between the two (P = 0.08) (Table 1).

**Table 1. The mean age difference between patients and controls by independent unpaired t-test**

| Group | Frequency | Mean age (years) | SE | SD | Range | Minimum | Maximum | P value |
|---|---|---|---|---|---|---|---|---|
| Patients | 50 | 44.49 | 1.61 | 11.36 | 47 | 22 | 69 | 0.08 |
| Controls | 30 | 48.39 | 1.49 | 8.16 | 30 | 36 | 66 | |

The patients' group where further subdivided according to the age into five different groups; 20-30 years old group represented 12% of the patients, 30-40 years, 40-50 years, 50-60 years, and 60-70 years' age groups were resembled by 28%, 26%, 26%, and 8%, respectively (Figure 2).

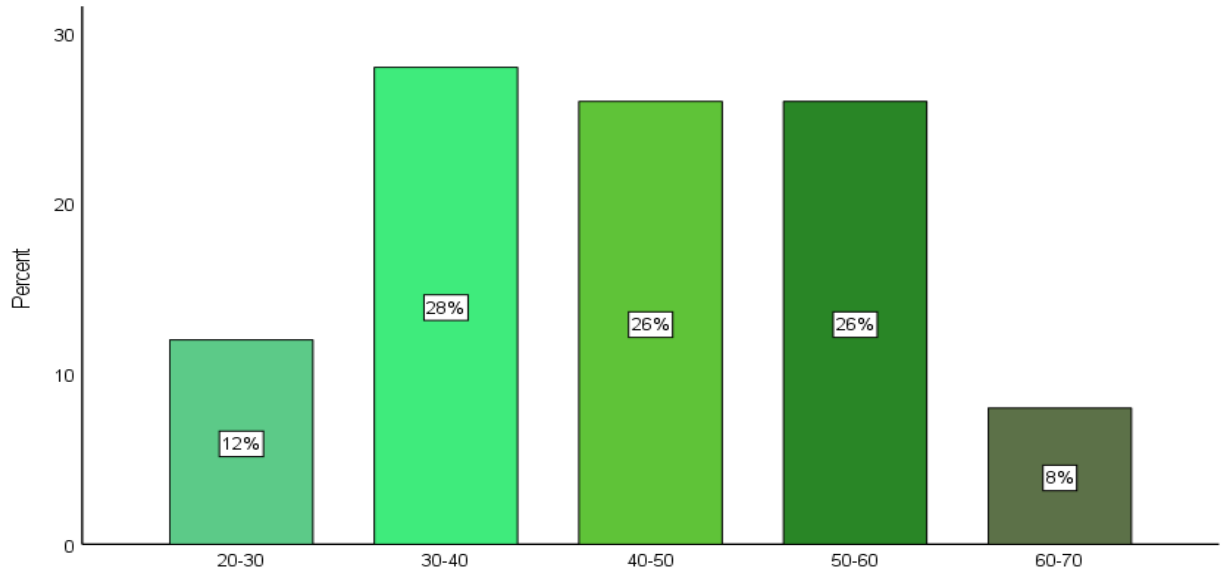


**Figure 2. The distribution of patients into age groups**

In the control group, the subjects were assigned into four age groups; those between 30-40 years old, between 40-50 years old, between 50-60 years old, and between 60-70 years old. Their percentages of the whole group were in the order of 23.3%, 36.7%, 30%, and 10% (Figure 3).

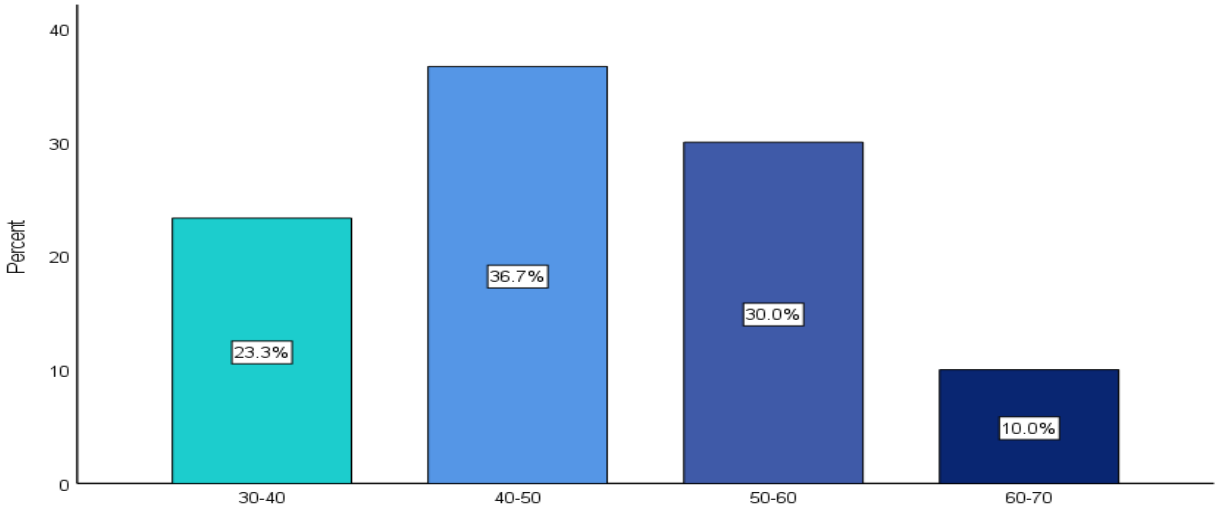


**Figure 3. The distribution of controls into age groups**

The results of the present work showed that the four biomarkers devoted for the diagnosis of infection with *H. pylori* (*H. pylori* IgA and *H. Pylori* IgG) and the determination of inflammatory response (IL-6 and TNF-α were differing very highly statistically significantly (P <0.001) when their mean serum levels of patients were compared to those of the controls. The mean serum levels of the IgA, IgG, IL-6, and TNF-α for the patients were as follows: 32.56±0.51 U/ml, 18.37±0.39 U/ml, 71.05±1.09 pg/ml, 54.72±1.01 pg/ml while those of the controls were: 7.97±0.35 U/ml, 2.09±0.21 U/ml, 11.69±0.50 pg/ml, 3.76±0.23 pg/ml (Table 2).

**Table 2. Independent unpaired t-test comparison results of the mean serum concentrations between patients and controls**

| Group | Frequency | Mean | SE | SD | P value |
|---|---|---|---|---|---|
| Patients | IgA | 32.56 U/ml | 0.51 | 3.59 | <0.001** |
| Controls | | 7.97 U/ml | 0.35 | 1.93 | |
| Patients | IgG | 18.37 U/ml | 0.39 | 2.79 | <0.001** |
| Controls | | 2.09 U/ml | 0.21 | 1.13 | |
| Patients | IL-6 | 71.05 pg/ml | 1.09 | 7.71 | <0.001** |
| Controls | | 11.69 pg/ml | 0.50 | 2.74 | |
| Patients | TNF-α | 54.72 pg/ml | 1.01 | 7.14 | <0.001** |
| Controls | | 3.76 pg/ml | 0.23 | 1.25 | |

**Very highly statistically significant at P <0.001

Analysis of variance (one-way ANOVA) was applied to determine the nature of the differences in the mean serum concentrations of IgA, IgG, IL-6, and TNF-α among age groups of the patients. Table 3 shows that the mean serum concentrations of the IgG only showed very highly statistically significant difference (P <0.001) while all other comparisons indicated no statistically significant difference (P = 0.76, P = 0.76, P = 0.70) for the IgA, IL-6 and TNF-α comparisons, respectively. The Bonferroni Post Hoc analysis was also applied to specify the age group that differed statistically significantly in term of the mean serum concentrations of the IgG, it was demonstrated that all age groups of the patients had statically significant difference among each other (P <0.001).

**Table 3. ANOVA test results for comparing studied parameters according to age groups of patients**

| Factor | Comparison | Degree of freedom | F value | P value |
|---|---|---|---|---|
| IgA | Between Groups | 4 | 0.473 | 0.76 |
| IgG | Between Groups | 4 | 169.696 | <0.001** |
| IL-6 | Between Groups | 4 | 0.473 | 0.76 |
| TNF-α | Between Groups | 4 | 0.550 | 0.70 |

**Very highly statistically significant at P <0.001

## Discussion

*H. pylori* infection causes chronic gastritis, which can evolve into severe gastroduodenal pathologies, as well as peptic ulcer, gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma. *H. pylori* is usually spread in childhood and perseveres for life if left untreated. Invasive, endoscopy-based and non-invasive methods, including breath, stool and serological tests, are used in the finding of *H. pylori* infection. Their use depends on the specific individual patient history and local availability [10].

The results of this work are in line with these of another study performed by a group of researchers (Castillo-Montoya, Ruiz-Bustos, Valencia-Juillerat, Álvarez-Hernández and Sotelo-Cruz) [11]. who showed that males (54%) were usually more susceptible to infection with *H. pylori* than females (46%), moreover same finding was also informed by Ertem et al. who performed their studies in Istanbul, Turkey and observed that 51.7% of the infected were males while females constituted 48.3% of all cases [12] In fact, there is a common understanding that males dominate the infection prevalence with *H. pylori*. A meta-analysis of 18 significant studies undertaken in adult populations shows the male preponderance of *H. pylori* infection, which was found to be homogenous and Consistent among populations from various countries. It was discovered that men are on average 16% more often infected by *H. pylori* than women, and this variance is statistically significant. These discoveries may partially explain the male predominance of *H. pylori-related* adult diseases, like duodenal ulcer and gastric adenocarcinoma [13].

In the present study, findings support the outcomes of other studies, which indicated sharp increase in disease prevalence in adolescence compared to other age groups [14-16]. Middle-aged individuals are more likely to get *H. pylori* infection due to a variety of factors including their lifestyle, immune response alterations, and cumulative exposure over time [17,18].

This study's findings are consistent with recent research that found a link between *H. pylori* infection and changes in serum immunoglobulin levels, particularly IgA and IgG. Elevated serum immunoglobulin levels are frequently symptomatic of the host immunological response to *H. pylori* infection and may serve as

possible biomarkers for disease diagnosis and surveillance [19-21].

The existence of *H. pylori* activates local and systemic cytokine signaling, which may disturb processes such as healing, gastric or duodenal rupture, and carcinogenesis [22]. The current study's findings are consistent with those of others, who have shown that serum TNF-α levels rise in patients with *H. pylori* infection. The overexpression of this cytokine, caused by this microbe, is also involved in the initiation of cancer and the advancement of gastric neoplasia [23,24]. It has been argued that the variation of serum concentrations of TNF-α is not statically significantly different between *H. pylori* positive and negative individuals, a statement that might contradicts the findings of this study; it appears that cells secreting this cytokine (e.g. macrophages) are primarily subverted in gastric mucosal tissue and as well soluble TNF receptors (sTNF-Rs) in serum, reliant on its concentration, may in some circumstances impede the outcomes of TNF-α [25].

A previous study evaluated inflammatory markers, including in IL-6, in individuals affected by *H. pylori* compared to healthy controls, demonstrating higher levels of this biomarker among cases suffering this bacterium. This suggests that chronic inflammation might contribute to *H. pylori*-related diseases by exacerbating damage and destroying tissue [26]. The latter conclusion is in complete agreement with the result of the present study.

In conclusion, infection with *H. pylori* can significantly alter the diagnostic markers and the markers commonly used to determine post-infection inflammatory response thus those markers can be utilized to diagnose and/or follow up infection progress.

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## Conflict of interest

There is no conflict of interest.

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