BioMed Visions Journal

Volume 2 | Issue 1 Article 8

1-15-2026

Helicobacter pylori Infection's Impact on Thyroid Dysfunction and Serum Interleukin-6 Levels

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Recommended Citation

Sehree, Mohamed M. and Al-Safar, Ashraf R. Salem (2026) "*Helicobacter pylori* Infection's Impact on Thyroid Dysfunction and Serum Interleukin-6 Levels," *BioMed Visions Journal*: Vol. 2: Iss. 1, Article 8.
DOI: https://doi.org/10.63100/3078-6738.1019

BIOMED VISIONS JOURNAL 2026;2:54–62

ORIGINAL STUDY

Helicobacter pylori Infection's Impact on Thyroid Dysfunction and Serum Interleukin-6 Levels

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ABSTRACT

Helicobacter pylori is a Gram-negative bacterium associated with peptic ulcer disease and gastric cancer. The study aimed to evaluate its prognosis and its relationship to sex, age, thyroid function, IL-6, and blood groups. 120 blood samples were collected from patients with gastrointestinal disorders at Al-Salam Hospital in Mosul between May and October 2024. The samples underwent clinical examination, including anti-H. pylori antibodies (chromatography and IgA ELISA to confirm active infection), thyroid hormones (Minvidus), interleukin-6 (ELISA), and blood typing using serological techniques.

The study results revealed that the rapid test revealed 40 positive cases out of 120, while the ELISA (IgA) test revealed 36 out of 40. The two approaches did not vary statistically substantially ($p = 0.346$), but the difference between positive and negative samples when using both methods was significant ($p = 0.0001$). The frequency of disease among males and females seemed to differ significantly ($p = 0.004$), with the infection being higher among females (72.2%). A significant difference was also found when comparing infected males ($p = 0.002$) and females ($p = 0.024$) to the control group. Regarding thyroid disorders, statistically significant differences ($p = 0.0001$) were found between individuals infected and uninfected with *H.pylori*. The incidence of thyroid disorders (hyperthyroidism and hypothyroidism) in the *H. pylori*-infected group was 47.2 % (17 out of 36), compared to 16.7 % (6 out of 36) in the uninfected group. Individuals with *H. pylori* infected showed a substantial rise ($p = 0.0001$) in IL-6 levels, with a mean of 35.76 ± 15.46 pg/ml compared to 4.03 ± 4.85 pg/ml in uninfected patients. Blood group A was more common among infected patients than among uninfected patients, with a statistically significant difference ($p = 0.0001$).

The present investigation discovered a clear connection between elevated IL-6 concentrations and thyroid issues and the bacteria *H. pylori* infection.

Keywords: *H-pylori*, ELISA, Hyperthyrodisim, Hypothyrodisim, Interlukin 6

1. Introduction

Helicobacter pylorus (*H. pylori*) is known like a spiral shape, gram-negative G^{-ve} and micro aerobic (Guo et al., 2021), dimensioned as 0.5 to 1 μm wide, 2 to 4 μm long, described as a short helical S-shaped microorganism (Öztekin et al., 2021), with from 3 to 5 polar flagella which deliver motility, has catalase, urease, and oxidase enzyme (Piscione et al., 2021). *H. pylori* have several factors which enable it for resisting a pH of 2.0 and could survive in the gastric environment (Keikha, 2020). *Helicobacter pylori* bacteria are found in the lower part of the stomach, and because of their shape and appearance, they can damage the stomach lining.

One of the most prevalent chronic bacterial illnesses, *Helicobacter pylori* (*H. pylori*), is more likely to affect humans. It is found all over the world and is an essential contributor to several illnesses, such as severe heart disease, psoriasis, cancer of the gut, autoimmune thyroid, prostatitis, heart attack, liver disease, and duodenal and stomach ulcers. *H. pylori* currently infects more than 50% of the world's populations, and in the last decade, it has been recognized

Received 21 July 2025; revised 1 September 2025; accepted 19 September 2025.
Available online 15 January 2026

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https://doi.org/10.63100/3078-6738.1019

as one of the most common chronic bacterial infections worldwide (Omar, Hassan, and Hasan, 2019; Lateef, 2023).

Accurate diagnosis of *Helicobacter pylori* is essential for managing gastroduodenal diseases. Various invasive and non-invasive tests exist, each with specific advantages and limitations. While no single gold standard exists, multiple reliable methods have been developed. Invasive tests involve endoscopic biopsies, including histology, culture, rapid urease test, and molecular techniques. Advances in endoscopy aid in real-time diagnosis. Non-invasive tests like the urea breath test and stool antigen test are widely used, while serology is mainly for screening and epidemiological purposes (Wang et al., 2015).

Thyroid disorders are among the most common endocrine diseases in the world, affecting one of the most important glands. These hormonal imbalances can lead to major digestive problems (Daher et al., 2009). The main thyroid disorders are hypothyroidism—characterized by insufficient production or action of T3 and T4 hormones (Patil, 2014) and hyperthyroidism, which results from excess thyroid hormones due to increased synthesis or external sources (Kravets, 2016).

This infection has been linked to other health issues, like autoimmune thyroid diseases, including autoimmune atrophic thyroiditis, Hashimoto's thyroiditis, and primary hyperparathyroidism, but the information from different sources is not consistent (Mokhtariye et al., 2019). However, a number of research efforts have demonstrated a strong positive correlation between autoimmune thyroid disorders and *H. pylori* infection (Raafat et al., 2019). Given this potential connection, this study seeks to determine the connection and occurrence of thyroid-related disorders in individuals with an infection caused by *H. pylori*. The root causes of *H. pylori*-associated diseases remain unclear, despite extensive research into their pathogenesis. It is hypothesized that immunological and biochemical factors may contribute to the development of these conditions (Hussein and Ali, 2020). Consequently, interest has increased in identifying new biomarkers associated with *H. pylori* infection, which may shed light on its pathogenesis (Matsuoka and Yashiro, 2018). Inflammatory indicators like C-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and white blood cell (WBC) count have been assessed in particular research in individuals with *H. pylori* infection compared to those who do not have the problem. However, these studies differed in the levels of these markers in *H. pylori*-infected patients (Saito et al., 2000). Therefore, we found it necessary to address this relationship in our current study.

2. Method and material

2.1. Sampling

The cross-sectional study was conducted on 120 blood samples randomly collected from patients presenting to the Internal Medicine Department at Al-Salam Hospital in Mosul, Iraq, who were suffering from gastrointestinal disorders and pain. Patients underwent a clinical examination by a specialist physician. The sample collection period extended from May to October 2024. The samples were processed by placing them in gel tubes, obtaining serum, and freezing them at –20 ˚C until use.

2.2. Using rapid immunochromatography to identify H. pylori infection

All 120 samples were screened using the *Helicobacter pylori* (*H. pylori* Ab) test strip from Biotech China. This is a rapid, optical immunoassay for the quantitative detection of IgM antibodies to *H. pylori* in human whole blood, serum, or plasma samples.

2.3. Using Alegria®Anti-Helicobacter pylori IgA antibodies

The Aligria technology was used to detect IgA antibodies only on the 40 samples that tested positive using the rapid method to identify early infection within these samples Alegria® Anti-*Helicobacter pylori* IgA is an automatic within-vitro assay device that uses ELISA to measure the amount of antibody type IgA anti the pathogen *Helicobacter pylori* in human serum as well plasma.

Based on the above results regarding the *Helicobacter pylori* infection test, the 36 positive samples were considered (the infection group), while 36 samples were randomly selected from among the 80 samples (which showed negative results for *H. pylori* infection). Thus, we had two groups (the infection group and the control group) that were subjected to thyroid hormone and interleukin-6 levels, in addition to blood group analysis.

2.4. Using Roche Cobas 6000-e602 to estimate thyroid hormones

Triiodothyronine (T3), tetraiodothyroxine (T4), and thyroid stimulating hormone (TSH) quantities were determined utilizing an electrochemiluminescent immunoassay (ECLIA) made for automation immune assays (Roche Cobas 6000-e602; Roche Diagnostics, Germany) for the purpose to identify problems with the thyroid.

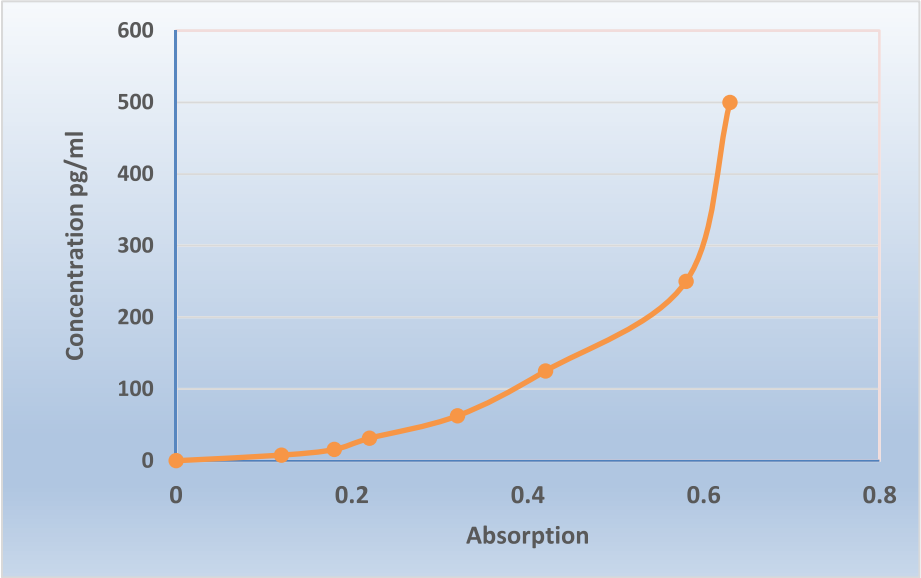

Fig. 1. Standard curve of interleukin 6.

2.5. Using ELISA to estimate human Interleukin 6 (IL6) in serum

A human IL6 (interleukin 6) levels were measured using a human IL6 ELISA kit (ELK Biotechnology, China) based on a sandwich immunoassay.

To create a typical curve, the average absorption of the standards was graphed versus the concentrations they had. The amount of IL-6 present was ascertained by matching each sample's optical density (OD) to the aforementioned curve, as illustrated in Fig. 1.

3. Results and discussion

3.1. Diagnosis of Helicobacter Pylori infection

As a result of their heightened cost and lengthy duration, invasive diagnostic methods are unsuitable for all patients who visit various governmental or private clinics for gastrointestinal troubles. Therefore, noninvasive strategies are preferred due to their speed and availability. In this study, both rapid techniques and the ELISA were employed.

As shown in Table 1, the rapid test (IgM strip) for *H. pylori* infection resulted in 40 (33.33%) positive samples out of 120. The ELISA test (IgA antibodies) yielded a positive result for only 36 (90 %) of the 40 samples However, there was no discernible statistically significant change for the number of positive samples between the two methods, with a probability value of 0.346. Nevertheless, the statistical program showed a clear difference, with a probability value of 0.0001, between the number of positive and negative samples when the two analytical methods were used separately.

Table 1. Diagnosis of *Helicobacter Pylori* Infection using two different methods.

	Positive No. (%)	Negative No. (%)	P. value
Rapid cassette	40 (33.33%)	80 (66.66%)	0.0001
Anti IgA Ab.by ELISA	36 (90 %)	4 (10%)	0.0001
P. value	0.346		
Total	120 (100%)		

*Results interpreted by Chi-Square test. Statistical test was done after considering the P-value = 0.05.

The result of the current study is similar to that reported by (Lateef, 2023). In 1993, Mansikka reported that only 80% of the samples were IgA antibody positive, indicating that many patients may be in an active phase of the infection (Mansikka et al., 1993). In 2018, Luisiniari noted that 20 % of IgA-negative cases may have had a previous infection, or their immune systems may not have produced enough IgA to detect the test (Loesnihari, 2018).

3.2. The influences of gender on H. pylori

Table 2 and Fig. 2 indicate a significant difference (P value = 0.004) in the distribution of positive samples regarding *Helicobacter pylori* infection between males and females. The table reveals that females accounted for the majority of infection cases, with a rate of 72.2%. The difference was also significant and precise at the time regarding the number of infected individuals versus controls in males and females, with P values of 0.002 and 0.024, respectively.

Table 2. Patient incidence for *H. pylori* based on gender.

H. pylori antibody test by Immunochromatography			
	Total Test No. 120 (100%)		
Gender	Total Positive 36 (100%)	Total Negative 84 (100%)	P. value
Male	10 (27.8%)	33 (39.3%)	0.002
Female	26 (72.2%)	51 (60.7%)	0.024
P. value	Total Positive 36 (100%)	Total Negative 84 (100%)	

* Results interpreted by Chi-Square test. Statistical test was done after considering the P-value = 0.05.

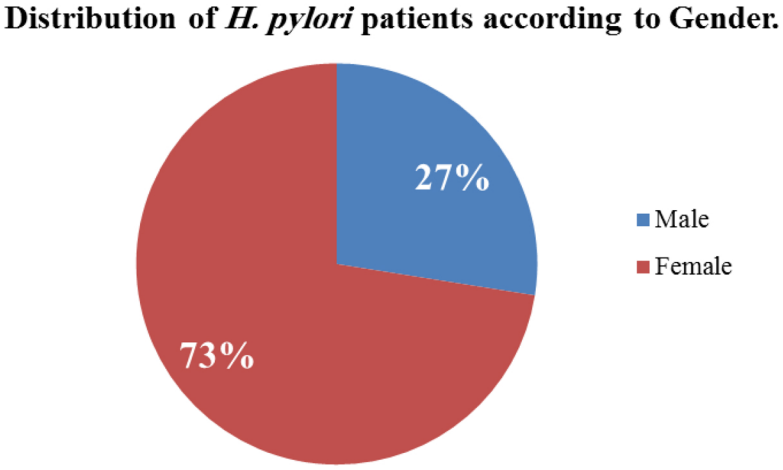

Fig. 2. Comparison of anti-*H. pylori* antibody frequency between male and female patients.

According to Table 3 and Fig. 3, the life expectancy of men and women did not differ substantially appreciably (p-value = 0.239) infected with *H. pylori*. The table below showed that the life expectancy was 34.45 ± 10.66 and 36.62 ± 12.71 for males and females, respectively. Furthermore, the data demonstrated that the mean age of infected and uninfected people did not differ statistically considerably for both males and females, with p-values of 0.318 and 0.898, correspondingly.

Regarding the above results, our current study aligns with the findings of the researchers Majeed and Khoshnaw, Kadhim, and AL-Hammam (Majeed and Khoshnaw, 2020; Al-Daamy, 2022; Kadhim and AL-Hammam, 2024). Nevertheless, our findings differed markedly from those of many other studies, such as the study performed by De Martel and Parsonnet, which investigated the effect of gender as a risk factor for infection with *Helicobacter pylori* (De Martel and Parsonnet, 2006). Almashhadany (2018) also discovered that men had an elevated prevalence of H pylori. The difference in hormones between the sexes and the fact that women are more likely than men to take care of food preparation and spending a longer period in the kitchen and housekeeping may be the cause of the greater infection rate among the women in our study (Mohammed, 2017), except for the difference in the mean ages of infected and uninfected males, which

Table 3. Categorization of H. pylori cases according to age groups.

H. pylori antibody test by Immunochromatography			
	Test No. (%)		
Gender	Total Positive 40 (100%) (Mean ± SD) Years	Total Negative 80 (100%) (Mean ± SD) Years	P. value
Male	34.45 ±10.66	39.09 ± 14.92	0.318
Female	36.62 ± 12.71	37.0 ± 12.54	0.898
P. value	0.239	0.119	

* Results interpreted as mean ± SE and p-value corresponding to Paired Samples T-test., Statistical test was done after considering the P-value = 0.05.

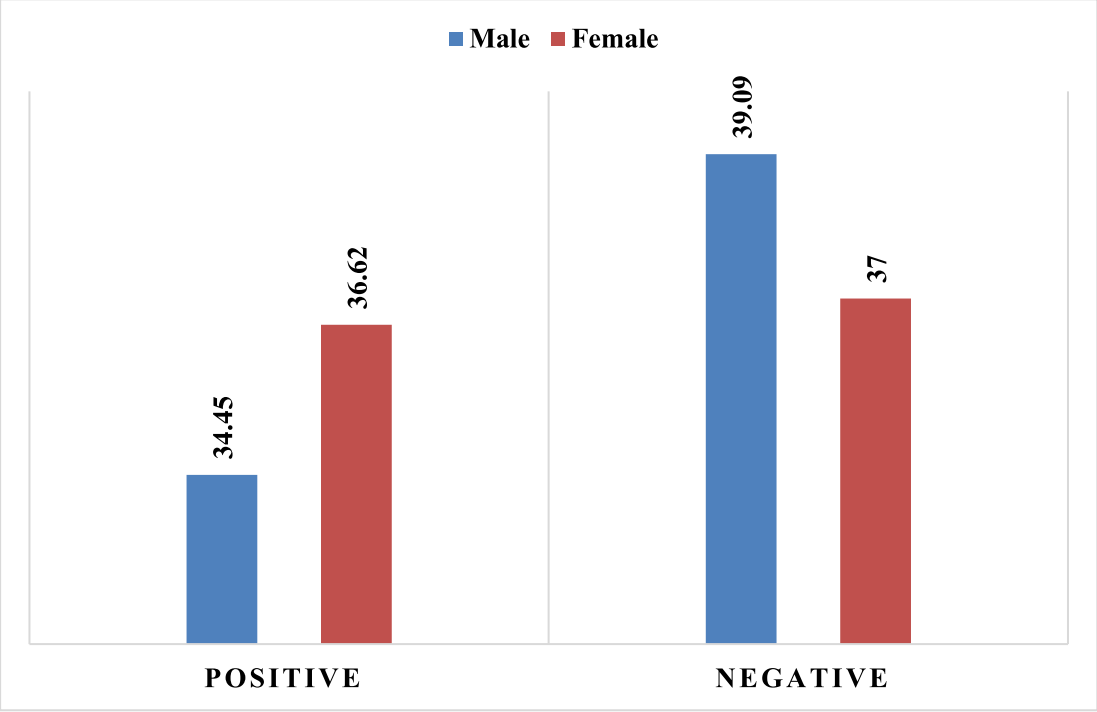

Fig. 3. Mean ages of males and females: Comparison between infected and uninfected.

was statistically significant with a p-value of 0.0409, as reported in the aforementioned research.

3.3. The influence of H. pylori infection on thyroid conditions

Table 4 and Fig. 4 indicates statistically significant differences between individuals infected with and without *H. pylori* with regard to thyroid disorders. The incidence rates of hyperthyroidism and hypothyroidism, as well as the incidence of healthy individuals, were compared between the two groups. The incidence of hyperthyroidism was 13.9% (5 of 36) in the group that tested positive for *H. pylori* and 5.6% (2 of 36) in the group that tested negative (uninfected), with a statistically significant variance (p-value = 0.0001). Concerning hypothyroidism, a statistically important distinction in infection rates between the two study groups was also noted. The infection rate reached 33.3% (12 out of 36) in the *H. pylori*-positive group, in contrast to 11.1% (4 out of 36) in the *H. pylori*-negative group. As for healthy individuals without thyroid disorders, the percentage was 52.8% (19 out of 36) in the positive group, while it reached 83.3% (30 out of 36) in the negative group, reflecting a statistically important change (p -value = 0.04).

Our present findings agree with those reported by Saqi and coworkers, who studied *H. pylori* patients in many private clinics in Kirkuk, whose infection was verified by a professional physician (Saqi, Nooruldeen, and Al-kadhi, 2023). The results of this study are also comparable to those of Šterzl et al. (2008), who discovered a connection between *H. pylori* infection and autoimmune thyroid disease (ATD) (Šterzl et al., 2008) and also found a significant relationship between *H. pylori* infection and hypothyroidism (Hammad et al., 2011). On the other hand, Tomasi et al. (2005) did not discover any connection between thyroid illness and *H. pylori* infection.

This infection influences the development of thyroid diseases in several ways; one is by triggering

Table 4. Effect of *H. pylori* infection on the thyroid disorder.

H. pylori status	Hyperthyroidism No. (%)	Hypothyroidism No. (%)	Healthy No.(%)	P. value
Total Positive No. 36 (100%)	5 (13.9%)	12 (33.3%)	19 (52.8%)	0.015
Total Negative No. (control) 36 (100%)	2 (5.6%)	4 (11.1%)	30 (83.3%)	0.0001
P. value	0.0001	0.0001	0.04	

* Results interpreted as mean ± SE and p-value corresponding to Paired Samples T-test and Chi-Square test.
Statistical test was done after considering the P-value = 0.05.

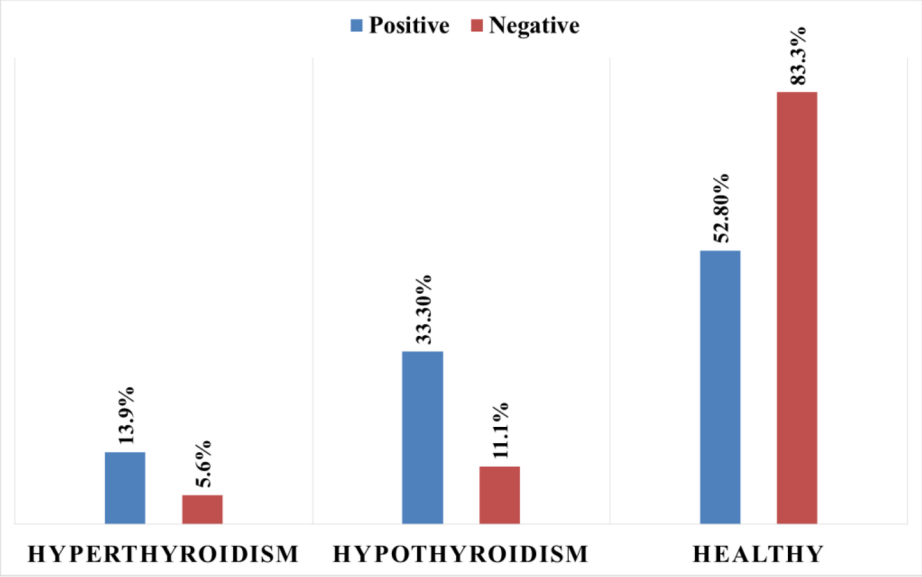

Fig. 4. Effect of *H. pylori* infection on the thyroid disorder.

an autoimmune reaction that may cause the immune system to become active. The immune system may be weakened by this continual stimulation, raising the possibility of autoimmune disorders like Graves' disease, which is the most common cause of hyperthyroidism, and Hashimoto's disease, which is the most common cause of hypothyroidism (Figura et al., 2020). Additionally, *Helicobacter pylori* infections can impact thyroid function by disrupting the absorption of nutrients and iodine (Muhsen and Cohen, 2008). *Helicobacter pylori* infection can also affect leptin and ghrelin secretion, subsequently affecting thyroid function (Barah, Al-quobaili, and Al-amouri, 2021).

3.4. The influence of H. pylori infection on interleukin-6 levels

Regarding interleukin-6 levels in patients infected with *Helicobacter pylori*, our findings, as presented in Table 5, demonstrated a significant, distinct, and statistically significant increase ($P < 0.05$) in this biomarker's concentration in the blood of those patients in comparison with the control group. Table 5 and Fig. 5 show that the levels of the biomarker in the blood of infected people are much higher (p value = 0.0001) than in healthy individuals, with an average of 35.76 ± 15.46 pg/ml in infected people compared to 6.03 ± 4.85 pg/ml in the healthy group.

In this study, the findings match those of Yu et al. (2023), who found that levels of IL-6, IFN-γ, and TNF-α went up after *Helicobacter pylori* infection, with the biggest increase in IL-6 seen in East Asia and the Middle East (Yu et al., 2023). An Egyptian study

Table 5. Effect of *H. pylori* infection on the Interleukin (IL6) level.

H. pylori status	Interleukin (IL6) pg/ml (*(Mean ± SD)*	P. value
Total Positive 36	35.76 ± 15.46	0.0001
Total Negative 36	6.03 ± 4.85	

* Results interpreted as mean ± SE and p-value corresponding to Paired Samples T-test.

also indicated that patients infected with *Helicobacter pylori* had higher serum IL-6 concentrations (Omar, Hassan, and Hasan, 2019). Another study by Nakagawa et al. found a strong link between higher IgG levels and increased IL-6 levels in patients with *Helicobacter pylori* (Nakagawa et al., 2013). Shubbar and Al-Buhamrah's research (2024) displayed mixed results and found no noticeable differences in IL-6 levels between the groups with and without *H. pylori* infection. Although the biomarker values in both groups were higher than the global normal, this increase was not statistically significant (Ebtehal and Shubbar, 2024). *Helicobacter pylori* infection causes higher levels of interleukin-6 (IL-6) in the blood because the immune system responds by activating immune cells and releasing inflammatory substances.

Table 6 and Fig. 6 delineate the correlation between blood groups and *H. pylori* infection. The table demonstrated a statistically significant disparity (p value = 0.0001) in blood group ratios between the two study cohorts (infected and non-infected with *H. pylori*). This was primarily represented by the significantly higher percentage of infected individuals with blood group A than the uninfected group.

Our analysis is compatible with the findings of a survey conducted in Gonabad, Iran, and the study

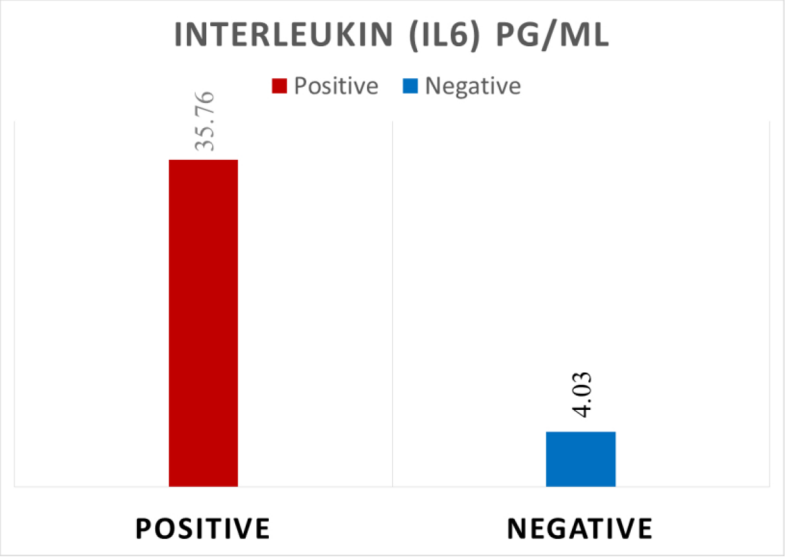

Fig. 5. Effect of *H. pylori* infection on the mean of Interleukin (IL6) level.

Table 6. Effect of *blood group* on the *H. pylori* infection.

Infected/uninfected *H. pylori*	Blood types				Rhesus (Rh) factor	
	A	B	AB	O	RhD -	RhD +
H. pylori Positive 36	3 (8.3%)	33 (91.7%)	12 (33.3%)	2 (5.6%)	7 (19.4 %)	15 (41.7%)
H. pylori Negative 84	4 (4.8%)	80 (95.2%)	33 (39.3%)	7 (8.3%)	19 (22.6%)	25 (29.8%)
P. value	0.0001					

* Results interpreted as mean ± SE and p-value corresponding to Chi-Square test. Statistical test was done after considering the P-value = 0.05.

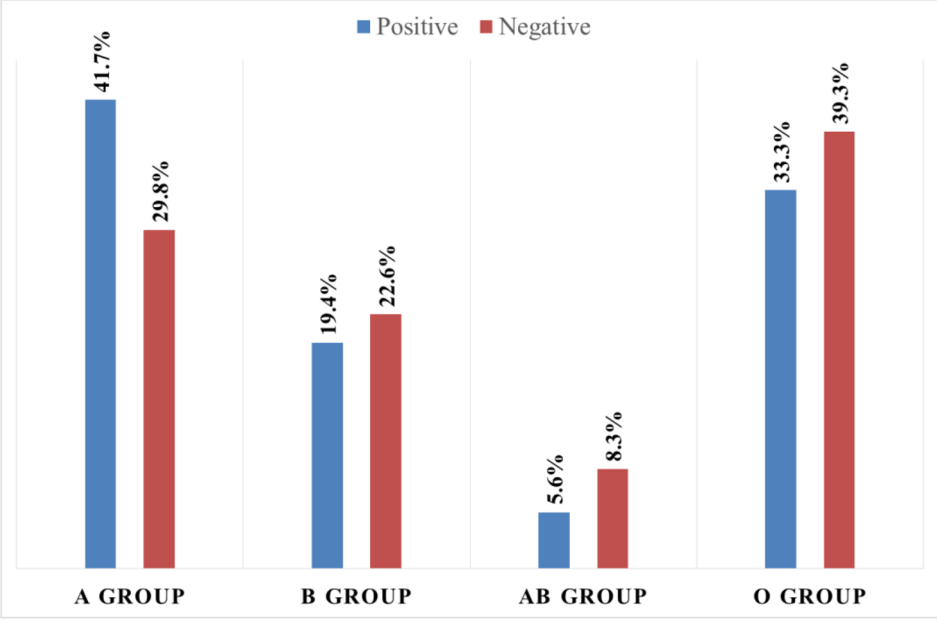

Fig. 6. Effect of *blood group* on the infected and uninfected with *H. pylori* infection.

by Kanbay et al. However, other studies have documented conflicting results. For example, Inoue et al. and Chakrani et al. reported that *H. pylori* infection in blood type O was higher than in other blood groups (Mohammadzadeh et al., 2023; Kanbay et al., 2005; Inoue et al., 2014; Chakrani, Robinson, and Taye, 2018). Other studies, such as Keller et al., Sharara et al., and Reiisi et al., have not observed any statistically significant relationship between blood groups and most *H. pylori* infections (Keller et al., 2002; Sharara et al., 2006; Reiisi et al., 2017).

4. Conclusion

The study results indicate that both the rapid IgG test and the ELISA (IgA) test are equally effective in detecting infection, with no significant difference between them. The results also showed a significant association between *H. pylori* infection and several factors, including gender, with females being more susceptible to infection. There was also a clear relationship between *H. pylori* infection and thyroid disorders, and elevated IL-6 levels. A significant relationship was also found between blood type and infection, with blood type A being more common among those infected.

Acknowledgment

We thank the management of Al-Salam Teaching Hospital in Mosul for facilitating the procedures for obtaining samples for the study.

Conflicts of interest

No any conflict of interest.

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