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Levels of Interleukins, Hepcidin and Ferritin in *Helicobacter pylori* Patients

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Abstract
The current study was aimed to estimate the prevalence of *H. pylori* in anemic patients and the levels of serum interleukins (1 and 6), hepcidin and ferritin. Totally, 110 individuals who went to the consulting clinic at Medical city Hospital in Baghdad Governorate during May to September (2022) were subjected to the current study. The blood samples were collected from the study population and the obtained sera were examined using the specific enzyme-linked immunosorbent assays (ELISA) to detect the prevalence of anti-*H. pylori* IgG-antibodies and the levels of interleukins, (1 and 6), hepcidin and ferritin. According to the results of serum analysis of 100 patients aged between 20-60 years, 75% were *H. pylori* positive. The percentage of males who tested positive was 62.7% compared to 37.3% females. The obtained results of hepcidin for anemia by *H. pylori* patients compared with the control group is 3.78 ± 0.17 ng/mL and 6.46 ± 1.52 ng/mL, respectively. It appeared that the hepcidin concentration was significantly lower ($p < 0.01$) for the patients than for the controls. The values of Interleukin-1B appeared to be higher for anemia by *H. pylori* patients compared to the control group. The data obtained from IL-1 showed a significant increase in the serum concentration of patients compared with the healthy control group ($p < 0.01$). As for values of Interleukin-6, the obtained results showed a significant increase in the serum concentration of patients compared with the healthy control group ($p > 0.05$). The values of interleukin-6 appeared to be higher for anemia by *H. pylori* patients. This study concluded that there is a relationship between low ferritin levels and infection with *H. pylori*, and this leads to cases of anemia in the patient. The high levels of cytokines showed a correlation with infection with *H. pylori*, as infection stimulates the immune system.While the levels of hepcidin protein did not show clear results and its relationship with infection with *H. pylori*. Because the serological tests do not distinguish between active infection and past exposure to *H. pylori*, further confirmation by other tests is required before eradication therapy. Several tests are available for the diagnosis of *H. pylori* infection. It should focus on the usefulness and limitations of each diagnostic method as well as the recent developments of these tests that contribute to improve the diagnostic accuracy. It should be considered in the future to extend current routine diagnostics of *H. pylori* infections with Next Generation Sequence

(NGS) applications. More researches are needed to establish different foods at high risk of *H. pylori* presence and transmission.
Keywords: Interleukin-1, Interleukin-1, Bacterial gastritis, Peptic ulcer, Iraq

Introduction

All nations are affected by the global health problem of anemia, but it is more common in countries with poor and medium incomes, in particular children aged 6 to 59 months who having the highest frequency of anemia (**Chaparro and Suchdev, 2019**). The World Health Organization (WHO) classifies anemia as a moderate public health issue when it affects 20-39.9% of people (**Kinyoki et al., 2021**). However, *Helicobacter pylori* in addition to numerous infections such as malaria, schistosomiasis, and chronic kidney disease impair the absorption of iron from diet (**Briguglio et al., 2020; Cappellini et al., 2020**). The primary factor contributing to anemia, which has other coexisting causes, is a deficiency in foods high in iron (both quantitatively and qualitatively), (**Diamond-Smith et al., 2016**). Contrary to popular belief, there are other nutritional factors that might contribute to anemia, such a shortage in folate, cobalamin, riboflavin, or vitamin A (**Bhadra and Deb, 2020**). Infections causes of anemia include parasitism, malaria, tuberculosis, and HIV. Cancer is one of the chronic diseases that can cause anemia. Inherited or acquired hemoglobin (Hb) synthesis errors, such as sickle cell disease, thalassemia, the presence of HbE, or disorders in erythrocyte production or survival, may also be causes of anemia in certain populations (**Brittenham et al., 2023**).

In clinical practice, inflammation is an organism's response to bacterial or viral infections, as well as the expression of the onset of chronic diseases and obesity (**Bennett et al., 2018**). During the inflammatory process, interleukins are released, which prompts the liver to release the hormone peptide hepcidin that works on the mononuclear phagocytic system to sequester circulating iron and prevents iron absorption from meals (**Nairz and Weiss, 2020; Mu et al., 2021**). In these circumstances, a combination of inadequate food absorption and poor body use results in iron deficiency with elevation in levels of C-reactive protein (CRP), alpha 1-acid glycoprotein (AGP), and other markers of chronic systemic (**Xu et al., 2017; Stoffel et al., 2021**). *Helicobacter pylori* is a gram-negative, spiral-shaped rod that is tiny, extremely motile, and has 4-6 unipolar sheathed flagella that confers and enables in viscous fluids, such as the mucus layer of the stomach epithelial cell, there is fast movement (**Vijay, 2018; Shreyas, 2019**). *Helicobacter pylori* affects approximately 50% of the population in their lifetime, is the cause of serious disease, and is classified as a class I carcinogen (**Miller and Williams, 2021**). This organism and anemia represent a significant global medical burden due to the sheer number of infected individuals, the chronic nature of infection, and the potential for severe illness outcomes (**Carpenter et al., 2010; Wertheim et al., 2018**). Therefore, the current study was aimed to estimate the prevalence of *H. pylori* in anemic patients and the levels of serum interleukins (1 and 6), hepcidin and ferritin.

Materials and methods

Ethical approval

The current study was licensed by the Scientific Committee of the Department of Microbiology in Collage of Veterinary Medicine (University of Ilam, Iran).

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Samples

Totally, 110 individuals who went to the consulting clinic at Medical city Hospital in Baghdad Governorate during May to September (2022) were subjected to the current study. The blood samples were collected from the study population and the obtained sera were examined using the specific enzyme-linked immunosorbent assays (ELISA) to detect the prevalence of anti-*H. pylori* IgG-antibodies and the levels of interleukins, (1 and 6), hepcidin and ferritin.

Serological assays

According to the manufacturer instructions of each specific kit (Shanghai, China), the Standard solutions of each ELISA's kit were calculated, and the number of stripes needed was determined by that of samples to be tested added by that of standards. It was suggested that each standard solution and each blank well should be arranged with three or more wells to be shacked gently and incubated at 37°C for 60 minutes. After removing the seal plate membrane carefully, the liquid was drained and the plate was filled with washing solution and drained after 30 seconds. This step was repeated for five times. A total 50 µl of Chromogen solution A was added firstly to each well and then 50 µl Chromogen solution B was added to each well as well. The plate was shacked gently and incubated for 10 minutes at 37°C. Then a 50 µl of Stop Solution was to each well to stop the reaction. Using the spectrophotometer, the absorbance (OD) was read at 450 nm wavelengths, and the concentrations were calculated based on the linear regression equation of the standard curve.

Statistical analysis

The t-test in the Microsoft Office Excel was applied to detect significant differences between values of study groups at $P<0.05$ (**Gharban et al., 2023**).

Results

According to the results of serum analysis of 100 patients aged between 20-60 years, 75% were *Helicobacter pylori* positive (Table 1).

Table (1): Results of *H. pylori* in study population

Results	Frequency (N)	Percentage (%)
Positive	75	75%
Negative	25	25%

The percentage of males who tested positive was 47 (62.7%), compared to 28 (37.3%) females (Figure 1).

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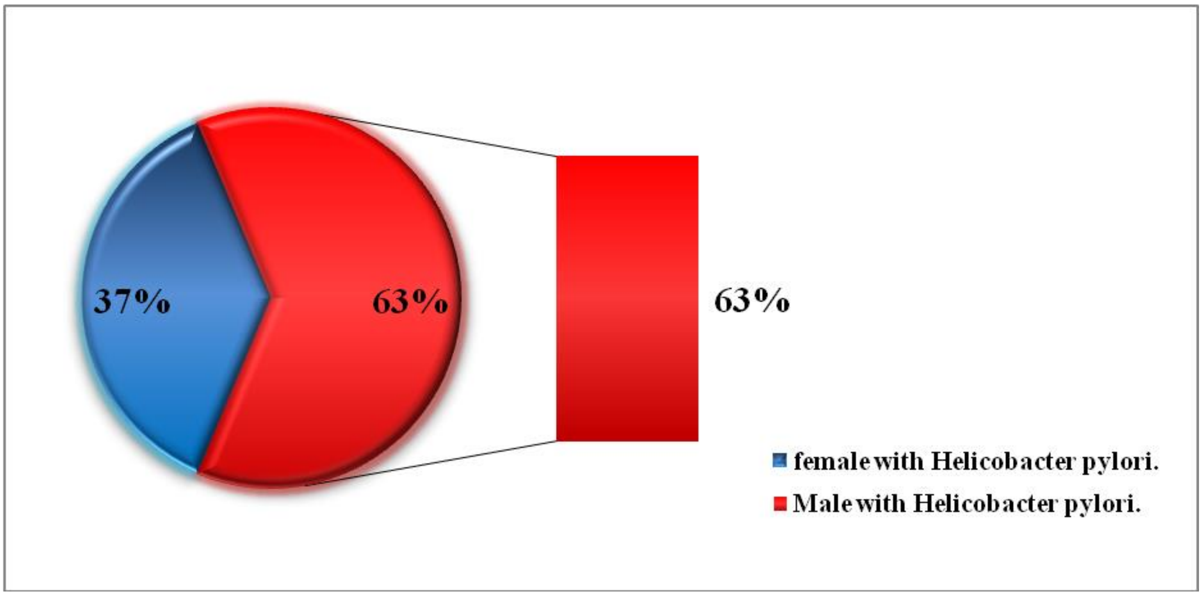

Figure 1: Distribution of infection according to sex of infected individuals

Serum levels of Interleukin-1, Interleukin-6 and hepcidin

The obtained results of hepcidin for anemia by *H. pylori* patients compared with the control group is 3.78 ± 0.17 ng/mL and 6.46 ± 1.52 ng/mL, respectively (Table 2, Figures 2-4). It appeared that the hepcidin concentration was significantly lower (p <0.01) for the patients than for the controls. The mean ± SD values of Interleukin-1B appeared to be higher for anemia by *H. pylori* patients compared to the control group; 83.24 ± 17.02 pg/ml and 32.79 ± 7.86 pg/ml, respectively. The data obtained from IL-1 showed a significant increase in the serum concentration of patients compared with the healthy control group (p <0.01). As for the values of Interleukin-6, the obtained results showed a significant increase in the serum concentration of patients compared with the healthy control group (p>0.05). The mean ± SD values of interleukin-6 appeared to be higher for anemia by *H. pylori* patients compared to the control group; 565.49 ± 177.48 pg/ml and 72.01 ± 14.91 pg/ml, respectively.

Table (4.3): Serum levels of immune markers and hepcidin in study individuals

Parameter	Patients (No: 75), M ± SD		Control (No: 25), M ± SD		P- value
Hepcidin	3.78 ±	0.17	6.46 ±	1.52	0.001
Interleukin-1	83.24 ±	17.02	32.79 ±	7.86	0.001
Interleukin-6	565.49 ±	177.48	72.01 ±	14.91	0.001
Ferritin	31.64 ±	3.72	125.88 ±	37.35	0.001

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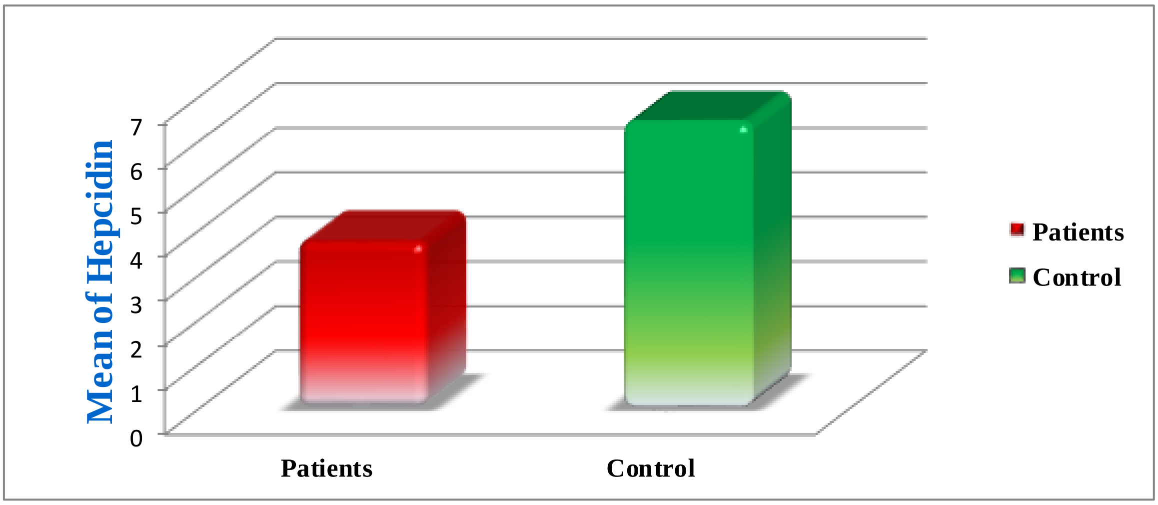

Figure 2: Level of serum IL-1 in study population

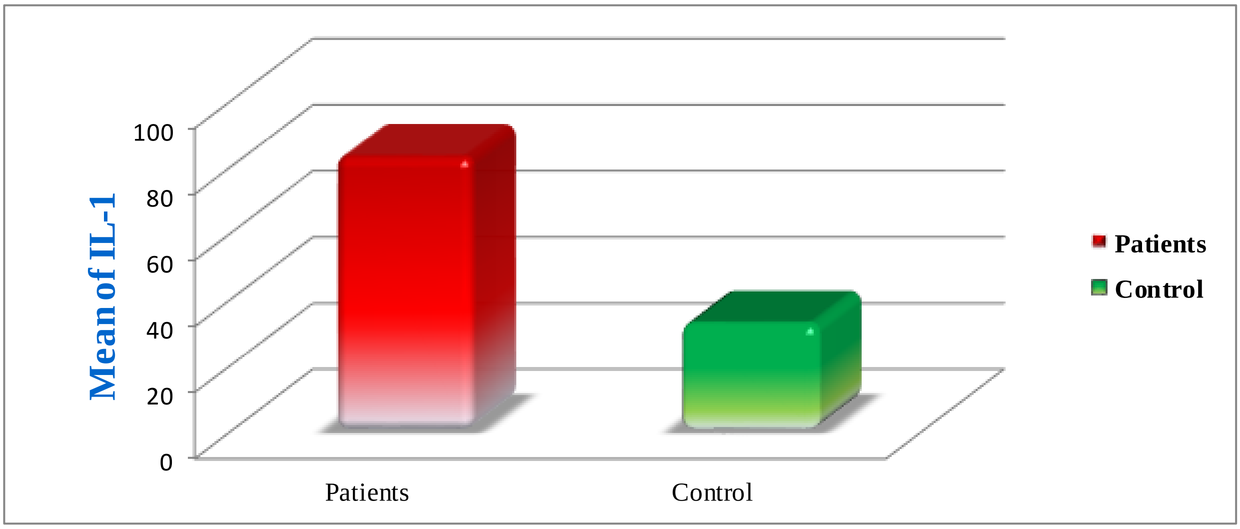

Figure 3: Level of serum IL-1 in study population

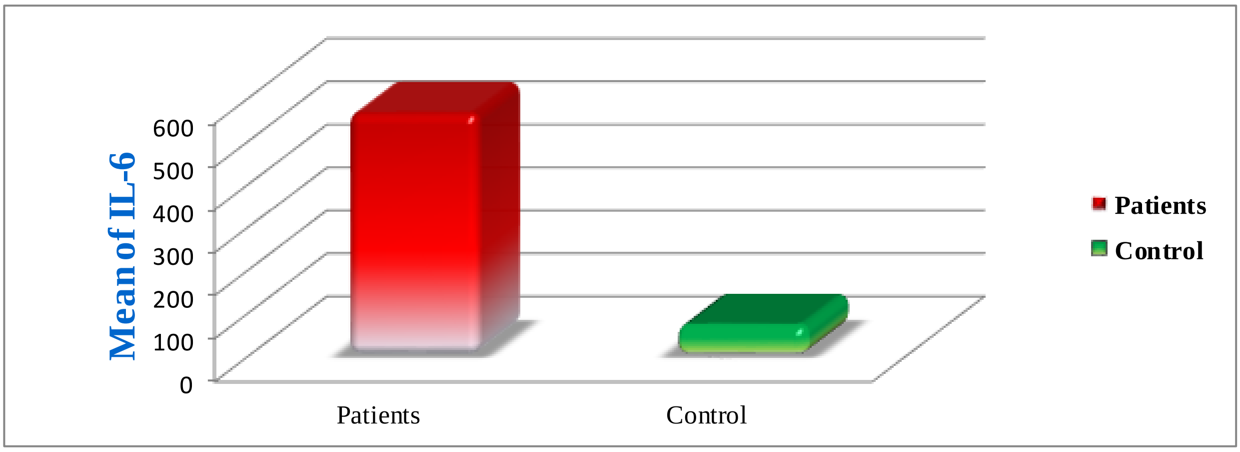

Figure 4: Level of serum IL-6 in study population

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Discussion

Our result is appeared that the hepcidin concentration was significantly lower ($p<0.01$) for the patients than for the controls. While the data obtained from Interleukin-1 showed a significant increase in the serum concentration of patients compared with the healthy control group ($p<0.01$). As for the values of Interleukin-6, the obtained results showed a significant increase in the serum concentration of patients compared with the healthy control group ($p>0.05$). During the progression of gastrointestinal diseases, a strong link was discovered between *H. pylori* and the host immune system (**Kumar and Dhiman, 2018**). In which, *H. pylori* induces the production of numerous pro-inflammatory cytokines such as interleukin-1 beta and interleukin-10 and tumor necrosis factor-alpha (**de Brito et al., 2018**). Interleukin-1b is up-regulated in the gastric antrum and corpus during the early stages of *H. pylori* infection, acts directly on gastric parietal cells to inhibit gastric acid secretion, and regulates the transcription of several proinflammatory cytokines, including Interleukin -6 (**Díaz et al., 2018; Song et al., 2020**).

Another study found that Interleukin-1b was elevated in the stomach of *H. pylori* positive patients but not in the serum of *H. pylori* negative patients with gastritis or ulcers (**Lu et al., 2005; Ramis et al., 2015**). This supports a previous theory that *H. pylori*-induced Interleukin -1b expression could lead to Interleukin-6 production and subsequent hepcidin expression (**Schwarz et al., 2012; Chen et al., 2018**). The role of hepcidin in the advancement of *H. pylori*-related iron deficiency has been widely debated, but it is still debatable (**Kato et al., 2012**). Recent research has also discovered increased gastric mucosa Interleukin-1b concentrations in people infected with *H. pylori*, which was found to be inversely related to blood ferritin levels (**Dunne et al., 2014; Figueiredo et al., 2014**).

Recent results for patients with ferritin deficiency and *H. pylori* compared with the control group the comparison was significant ($P \leq 0.05$), at 102.44 ± 63.62 and 69.89 ± 59.7 for patients and control groups respectively. This result means for our study, the patient had a decrease in the level of serum ferritin affected by *H. pylori* infection. Another study found that *H. pylori* infection can result in lower serum ferritin and higher total iron-binding capacity levels found that *H. pylori* infection causes a decrease in serum ferritin levels, which is consistent with our findings (**Tari et al., 2016; Kishore et al., 2021**).

Serum ferritin amounts were found to be elevated in *H. pylori*-infected individuals, possibly due to the inflammatory processes caused by *H. pylori* infection. This, however, contradicts a previous study in which eradication of *H. pylori* infection was associated with an increase in serum ferritin levels. Low serum iron levels are strongly linked to iron deficiency anemia, and impactful *H. pylori* infection management can help patients overcome anemia and raise iron stores for a higher standard of living (**Milman et al., 1998; Rahman et al., 2020**).

In one study, **Miernyk et al.** (**2013**) found that 121 out of 241 patients were *H. pylori*-positive, and a large percentage of infected people had iron-deficiency anemia compared to uninfected people. According to serum ferritin levels are lower in children with *H. pylori* infection, suggesting that *H. pylori* infection may result in iron deficiency in children (**Harris et al., 2013**). Many studies have found a link between *H. pylori* infection and low serum ferritin levels, but other studies have not confirmed this link (**Sato et al., 2015**). Through the results of the current research and other research, it was shown that the largest percentage of people infected with *H. pylori* suffer from a deficiency of the protein ferritin.

Conclusion

This study concluded that there is a relationship between low ferritin levels and infection with *H. pylori*, and this leads to cases of anemia in the patient. The high levels of cytokines showed a correlation with infection with *H. pylori,* as infection stimulates the immune system. The levels of hepcidin protein were not showed clear results about its relationship to *H. pylori* infection. Because the serological tests do not distinguish between active infection and past exposure to *H. pylori*, further confirmation by other tests is required before eradication therapy. Several tests are available for the diagnosis of *H. pylori* infection. It should focus on the usefulness and limitations of each diagnostic method as well as the recent developments of these tests that contribute to improve the diagnostic accuracy. It should be considered in the future to extend current routine diagnostics of *H. pylori* infections with Next Generation Sequence (NGS) applications. More researches are needed to establish different foods at high risk of *H. pylori* presence and transmission.

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