**ORIGINAL ARTICLE**

# Relationship between Hepcidin and Iron Deficiency Anemia in *Helicobacter pylori* Infected Patients

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## ABSTRACT

***Background:*** *Helicobacter pylori may be associated with unexplained iron-deficiency anemia (IDA). Hepcidin is an acute-phase reactant but its relation to H. pylori and IDA has not been elucidated. Objective: to investigate serum hepcidin in H. pylori infected patients and its role in IDA.* ***Methodology:*** *This study was performed on 70 patients infected by Helicobacter pylori. They were divided into two groups, group A: 35 H. pylori infected patients without iron deficiency anemia and group B: 35 H. pylori infected patients with iron deficiency anemia. Serum hepcidin was measured by ELISA in both groups while iron, ferritin and total iron binding capacity were estimated in group B.* ***Results:*** *Serum hepcidin was significantly higher in patients with H. pylori infection and iron deficiency anemia. However, non-significant correlation between hepcidin and both iron and ferritin were found.* ***Conclusion:*** *Helicobacter pylori could modulate serum hepcidin level in patients with iron deficiency anemia.*

## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) requires iron to survive and may potentially be associated with unexplained iron-deficiency anemia (IDA). *H. pylori* infection is more prevalent in patients with refractory IDA higher than in the normal people, and there had been an association reported between low serum ferritin and a high prevalence of *H. pylori*-specific IgG. Some cases with *H. pylori*-associated refractory IDA have been corrected by eradication of *H. pylori*, even without oral iron treatment[1].

IDA affects about 5% of the adult population in the developing world. It has been reported that *H. pylori* disrupts the iron regulatory mechanism via hepicidin. This 25-amino-acid peptide hormone first identified in human urine and plasma that is secreted mainly from the liver, exerts in vitro antibacterial and antifungal activities. Hepcidin induces disruption of the iron transporter protein ferroportin thus limiting release and absorption of iron. Hepcidin is an acute-phase reactant which expression is increased via interleukin (IL)-6 during bacterial infection and inflammation, but how this is related to *H. pylori* and IDA has not been fully elucidated, particularly in adults[2, 3].

The aim of our study was to measure the serum hepcidin levels in *Helicobacter pylori* infected patients and investigate its relation to IDA in those patients.

## METHODOLOGY

This study was carried out at Tropical Medicine (including the endoscopy unit) and Medical Microbiology and Immunology Departments, Faculty of Medicine, Zagazig University from November 2018 till November 2020.

**Inclusion criteria:**

This study was carried out on seventy patients infected by *Helicobacter pylori.* They were divided into two groups, group A: 35 *H. pylori* infected patients without iron deficiency anemia and group B: 35 *H. pylori* infected patients with iron deficiency anemia.

**Exclusion criteria:**

History of blood loss or positive occult blood in stool, pregnancy or lactation, severe systemic illness, manifested clotting disorders or the use of anticoagulants, history of blood transfusion or iron therapy during the past 6 months before study, presence of any evidence of other infections or inflammation and patients who had taken proton pump inhibitors or antibiotics through one month ago were excluded.

**Study design:**

This study was a case control study. All patients were subjected to the following:

- Proper history taking.
- Thorough clinical examination.
- Laboratory investigations include:
  - Liver and kidney function tests.

- Complete blood picture (CBC) (Sysmex XS, Japan). If CBC showed microcytic hypochromic anemia, iron profile (iron, ferritin, TIBC) was done.
- C-reactive protein.
  All clinical chemistry tests were carried out by cobas 8000 modular analyzer, Roche diagnostics.
- *Helicobacter pylori* antigen in stool was detected by using *H. pylori* Ag rapid test (CTK, USA).
- Occult blood in the stool was tested by rapid test (Abon Biopharm, China).
- Serum hepcidin (Hepc) was measured by using ELISA kit (Human Hepc ELISA kit (SunRed, China) according to manufacturer instructions and optical densities were measured under 450 nm wavelength by Biotek ELISA plate reader (USA).

▪ Upper GI endoscopy was performed using end flexible video-endoscope (PENTAX VIDEO unit of endoscopy) and gastric biopsies were taken for rapid urease test BIOHIT H. pylori quick test (Biohit Oyj, Finland).

**Statistical analysis:**

Analysis of data was done using Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median. Significance of the obtained results was determined at 5% level. The used tests were Chi-square test (for categorical variables, to compare between different groups), Student t-test (for normally distributed quantitative variables, to compare between two studied groups), Mann Whitney test (for abnormally distributed quantitative variables, to compare between two studied groups), Spearman coefficient (to correlate between two distributed abnormally quantitative variables). When a variable was not normally distributed, A P value < 0.05 is considered significant.

## RESULTS

As shown in table 1, in group A, there were 23 (65.7%) males, 12 (34.3%) females, with the mean age 32.66 (± 4.68 SD). In group B, there were 25 (71.4%) males, 10 (28.6%) females, with the mean age 33.57 (± 4.51 SD). There was no statistically significant difference between two groups for sex and age.

**Table 1: Comparison between the studied groups regarding demographic data**

| Demographic Data | Group A without IDA (n = 35) | | Group B with IDA (n = 35) | | Test of Sig. | P |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **Gender** | | | | | | |
| Male | 23 | 65.7 | 25 | 71.4 | $\chi^2$= 0.265 | 0.607 |
| Female | 12 | 34.3 | 10 | 28.6 | | |
| **Age (years)** | | | | | | |
| 25 – 30 | 13 | 37.1 | 9 | 25.7 | $\chi^2$= 1.070 | 0.586 |
| 30 – 35 | 9 | 25.7 | 11 | 31.4 | | |
| ≥ 35 | 13 | 37.1 | 15 | 42.9 | | |
| Min. – Max. | 26.0 – 41.0 | | 26.0 – 42.0 | | t= 0.832 | 0.409 |
| Mean ± SD. | 32.66 ± 4.68 | | 33.57 ± 4.51 | | | |
| Median (IQR) | 33.0 (28.0 – 36.50) | | 33.0 (29.50 – 37.50) | | | |

$\chi^2$: **Chi square test** **t: Student t-test** p: p value for comparing between the studied groups

As shown in table 2, there was a statistically significant difference between two groups as regards MCHC, MCH, MCV, Hb and RBCs.

**Table 2: Comparison between the two studied groups as regards CBC**

| CBC | Group A without IDA (n = 35) | Group B with IDA (n = 35) | Test of Sig. | P |
|---|---|---|---|---|
| **WBC n 4-11*10$^3$/mm$^3$** | | | | |
| Min. – Max. | 6.0 – 10.0 | 5.0 – 10.0 | t= 0.596 | 0.553 |
| Mean ± SD. | 8.03 ± 1.20 | 8.20 ± 1.21 | | |
| Median (IQR) | 8.0 (7.0 – 9.0) | 8.0 (8.0 – 9.0) | | |
| **Platelet n 150-400 *10$^3$/mm$^3$** | | | | |
| Min. – Max. | 300.0 – 400.0 | 300.0 – 398.0 | t= 1.225 | 0.225 |
| Mean ± SD. | 359.0 ± 25.05 | 365.9 ± 22.51 | | |
| Median (IQR) | 366.0 (337.0 – 377.5) | 368.0 (352.0 – 387.0) | | |
| **MCHC n 31-37 g/Dl** | | | | |
| Min. – Max. | 31.0 – 37.0 | 23.0 – 27.0 | t= 37.238* | <0.001* |
| Mean ± SD. | 35.0 ± 1.31 | 24.86 ± 0.94 | | |
| Median (IQR) | 35.0 (34.0 – 36.0) | 25.0 (24.0 – 25.0) | | |
| **MCH n 26-34 pg (picogram)** | | | | |
| Min. – Max. | 28.0 – 33.0 | 20.0 – 24.0 | t= 25.170* | <0.001* |
| Mean ± SD. | 30.03 ± 1.52 | 21.97 ± 1.12 | | |
| Median (IQR) | 30.0 (29.0 – 31.0) | 22.0 (21.0 – 23.0) | | |
| **MCV n 80-100 micro m$^3$** | | | | |
| Min. – Max. | 80.0 – 93.0 | 55.0 – 74.0 | t= 18.399* | <0.001* |
| Mean ± SD. | 86.80 ± 3.79 | 65.11 ± 5.85 | | |
| Median (IQR) | 87.0 (85.0 – 89.50) | 66.0 (60.0 – 69.5) | | |
| **RBC n 4.5-6 *100$^3$ / mm$^3$** | | | | |
| Min. – Max. | 4.0 – 5.60 | 2.50 – 3.80 | U= 0.00* | <0.001* |
| Mean ± SD. | 4.94 ± 0.34 | 3.19 ± 0.36 | | |
| Median (IQR) | 5.0 (4.65–5.10) | 3.30 (3.0–3.40) | | |
| **Hb n 13-17 g/dL** | | | | |
| Min. – Max. | 13.0 – 16.0 | 8.0 – 10.0 | t= 36.085* | <0.001* |
| Mean ± SD. | 14.43 ± 0.65 | 8.63 ± 0.69 | | |
| Median (IQR) | 14.0 (14.0 – 15.0) | 9.0 (8.0 – 9.0) | | |

**t: Student t-test** **U: Mann Whitney test**
p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$

Liver and renal functions were normal in both groups without significant difference. Table 3 describes levels of iron, TIBC, and ferritin in patients enrolled in group B. The mean total iron binding capacity (TIBC) was 457.9 (± 30.10 SD), the mean serum iron was 31.49 (± 1.12 SD), the mean serum ferritin was 15.09 (± 1.60 SD).

**Table 3: Iron profile in patients enrolled in in group B**

| Iron profile | Min. – Max. | Mean ± SD. | Median (IQR) |
|---|---|---|---|
| **TIBC (total iron binding capacity) n 250-410 micro g/dl** | 388.0 – 498.0 | 457.9 ± 30.10 | 467.0 (455.5 – 478) |
| **Serum iron n 60-160 micro g/dL** | 29.0 – 33.0 | 31.49 ± 1.12 | 32.0 (31.0 – 32.0) |
| **Serum ferritin n 25-280 ng/ml** | 12.0 – 19.0 | 15.09 ± 1.60 | 15.0 (14.0 – 16.0) |

As shown in table 4, In group A, the mean serum hepcidin level was 69.17 (± 130.34 SD) with range (21.0 – 787.0), and the mean C-reactive protein 3.89 (± 0.80 SD) with range (3-5). In group B, the mean serum hepcidin level was 93.60 (± 94.69 SD) with range (43.0 – 559.0), and the mean C-reactive protein was 3.77 (± 0.73 SD) with range (2-5). There was a statistically significant difference between the 2 groups regarding serum hepcidin level, while there was non-significant difference between both groups for CRP level.

**Table 4: Comparison between the studied groups regarding serum hepcidin and CRP**

| | Group A without IDA (n = 35) | Group B with IDA (n = 35) | Test of Sig. | P |
|---|---|---|---|---|
| **Serum hepcidin level in ng/mL** | | | | |
| Min. – Max. | 21.0 – 787.0 | 43.0 – 559.0 | U= 250.0* | <0.001* |
| Mean ± SD. | 69.17 ± 130.34 | 93.60 ± 94.69 | | |
| Median (IQR) | 35.0 (28.0 – 60.0) | 66.0 (54.0 – 88.0) | | |
| **C-reactive protein mg/L** | | | | |
| Min. – Max. | 3.0 – 5.0 | 2.0 – 5.0 | t= 0.626 | 0.534 |
| Mean ± SD. | 3.89 ± 0.80 | 3.77 ± 0.73 | | |
| Median (IQR) | 4.0 (3.0 – 4.50) | 4.0 (3.0 – 4.0) | | |

**t: Student t-test** **U: Mann Whitney test**
p: p value for comparing between the studied groups *: Statistically significant at $p \leq 0.05$

Figures 1, 2, 3 show that there was non-significant positive correlation between serum hepcidin level and TIBC, non-significant negative correlation between serum hepcidin level and serum iron, and non-significant negative correlation between serum hepcidin level and serum ferritin.

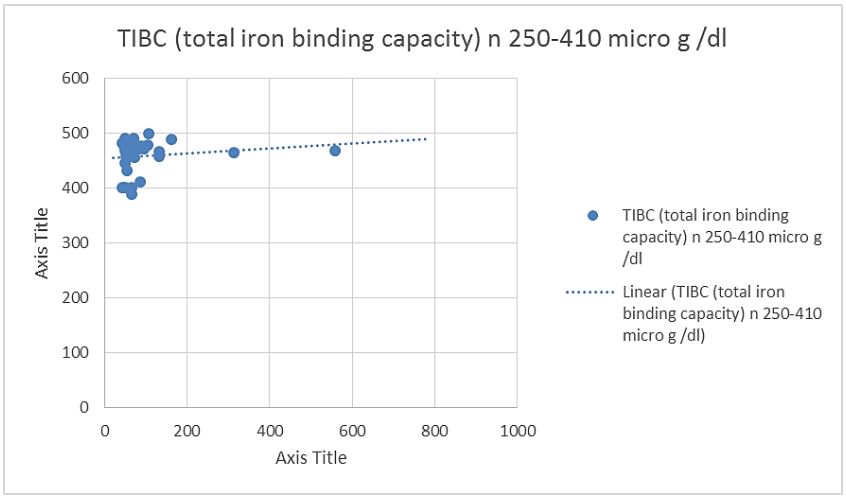


**Figure 1:** Correlation between serum hepcidin and TIBC (total iron binding capacity) in group B (non-significant positive correlation)

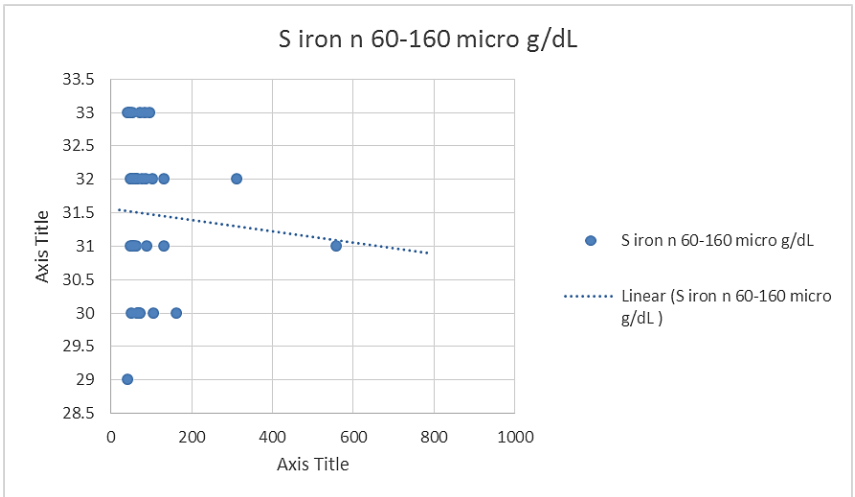


**Figure 2:** Correlation between serum hepcidin and iron in group B (non-significant negative correlation)

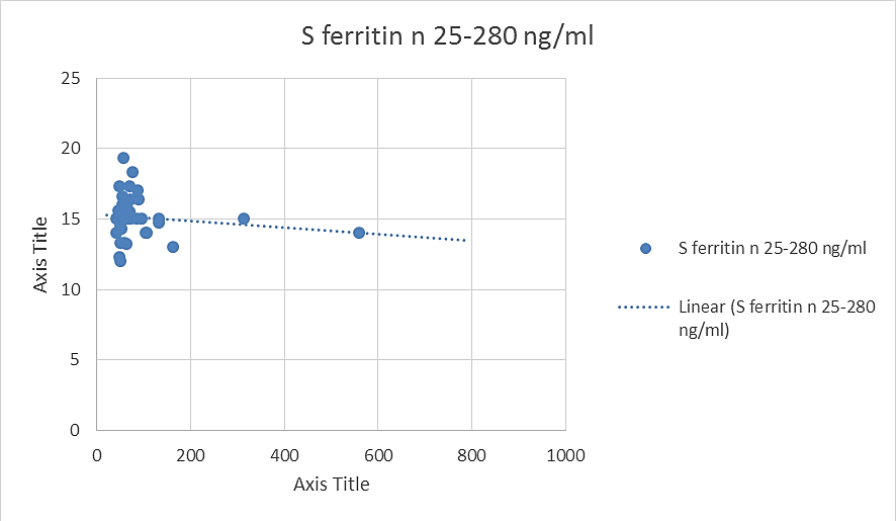


**Figure 3:** Correlation between serum hepcidin and ferritin in group B (non-significant negative correlation)

## DISCUSSION

Human hepcidin is secreted mainly from the liver and exerts in vitro antibacterial and antifungal activities. It is an acute-phase reactant which expression is stimulated by interleukin (IL)-6 during bacterial infection and inflammation[4]. In addition, hepcidin plays a major role in homeostatic regulation of iron metabolism. This peptide binds to the cellular iron exporter ferroportin causing its disruption, thus trapping iron in enterocytes, macrophages, and hepatocytes[5].

*Helicobacter pylori* infection has been reported as cause of iron deficiency anemia (IDA) in patients with unexplained IDA. Mechanisms include blood loss due to erosive gastritis, diminished iron absorption after atrophy-associated hypochlorhydria, and increased iron uptake by *Helicobacter pylori*[6]. The role of hepcidin in IDA in *H. pylori* infected people has not been fully investigated. Therefore, the goal of this study was to study the possible role hepcidin in IDA among *H. pylori* infected patients.

The study was carried out on 70 patients separated into 2 groups, group A included 35 patients with *H. pylori* infection and without IDA and group B included 35 patients with *H. pylori* infection and IDA. Statistical analysis of the 2 groups regarding age and sex showed that both groups were matched for age and sex.

In this study, both groups had normal liver functions without significant difference. This agrees with Peng et al.[7] who showed no significant difference in the levels of ALT, AST, ALP and GGT between *H. pylori*-positive IDA patients and *H. pylori*-positive non-IDA patients. On the other hand, Salehi et al.[8] showed correction in liver enzymes after eradication of *H. pylori*, suggesting a role for *H. pylori* infection in mild unexplained hypertransaminasemia.

In the present study, renal functions were normal without significant difference in both groups. However, Pan et al.[9] reported that *H. pylori* infection could be a risk factor for kidney damage and *H. pylori* eradication probably relieves kidney damage and prevents chronic kidney disease.

In this study, a highly significant difference between the 2 groups regarding serum hepcidin level was found; mean serum hepcidin level was 69.17 (± 130.34 SD) with range (21.0 – 787.0) in group A versus 93.60 (± 94.69 SD) with range (43.0 – 559.0) in group B. Such finding matches with Mendoza et al.[10] who found that in school children with *H. pylori* infection, hepcidin levels tended to be higher, regardless of the iron nutritional status. Also, Azab and Esh[11] conducted a study including 60 children with iron-deficiency anemia. Serum hepcidin was significantly lower in *H. pylori* noninfected children with IDA than *H. pylori* infected children with IDA. However, this was in disagreement with White et al.[2] who reported that serum hepcidin concentrations were significantly reduced in the *H. pylori* and IDA group compared to the control group which was *H. pylori* without IDA. The authors concluded that local or systemic factors such as inflammatory mediators could be driving this response as more severe atrophy was observed in IDA groups. Lee et al.[12] observed decrease in serum hepcidin levels after both *H. pylori* eradication and oral iron intake, with correction of IDA.

In the present study, there was non-significant positive correlation between serum hepcidin level and total iron binding capacity (TIBC), non-significant negative correlation between serum hepcidin level and serum iron, and non-significant negative correlation between serum hepcidin level and serum ferritin. These results agree with Hou et al.[13] who similarly found non-

significant association between *H. pylori* infection and serum iron and ferritin levels. Conversely, Azab and Esh[11] reported significant negative correlations between serum hepcidin and serum ferritin, Hb, iron, and transferrin saturation in *H. pylori*-infected children with IDA. Moreover, Qujeq et al.[14] found that total iron-binding capacity in *H. pylori* positive group was lower than in the control *H. pylori* negative group without IDA. The later results also disagree with Sapmaz et al.[15] who reported correlations between patient characteristics and rate of change in IDA parameters and serum hepcidin levels and found that the reduction rate of serum hepcidin level was negatively correlated with the rate of elevation in serum ferritin, iron, Hb, MCV and transferrin saturation values, while it was positively correlated with reduction rate in TIBC.

## CONCLUSION

*Helicobacter pylori* infection could modulate serum hepcidin level in patients with iron deficiency anemia.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

**Ethical considerations:**

- An informed verbal consent from all participants was taken and confidentiality of information was assured.
- An official written administrative permission letter was obtained from head of Tropical Medicine and Microbiology and Immunology Departments, dean of Faculty of Medicine, Zagazig university. The title and objectives of the study were explained to them to ensure their cooperation.
- Zagazig University International Review Board (IRB) #:1966/10-3-2015.

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