Hepcidin correlates with interleukin-1β and interleukin-6 but not iron deficiency in children with Helicobacter pylori infection

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Key Words
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Background: Helicobacter pylori infection is associated with iron deficiency (ID) in children. Inflammatory cytokine reactions could influence the consequences of H. pylori infection. Hepcidin is an important regulator in iron homeostasis and could be induced by chronic inflammation. The relationship between hepcidin and cytokine levels in children infected with H. pylori remains controversial.

Methods: Based on serology testing for anti-H. pylori IgG, participants (43 seropositive and 43 seronegative) aged 10–18 years were enrolled. Serum hepcidin levels and iron profiles, including iron, ferritin, and total iron-binding capacity, were measured. ID is defined as iron saturation less than 15%. Seropositive children were divided into low hepcidin (n = 22) and high hepcidin (n = 21) groups. IL-1β, IL-6, and IL-8 serum levels were compared.

Results: Serum IL-1β and IL-6 levels were comparable between H. pylori seropositive and seronegative children, as were the median serum hepcidin levels (6.5 ng/mL versus 8.6 ng/mL; P = 0.1318). Median levels of serum iron, ferritin, and iron saturation were significantly lower in seropositive children with low hepcidin than in those with high hepcidin (P = 0.0123, P = 0.0001, and P = 0.0004, respectively). The prevalence of ID was significantly higher in...
1. Introduction

*Helicobacter pylori* (H. pylori) is one of the most common chronic bacterial infections, affecting approximately half of the population worldwide.\(^1,2\) *H. pylori* infection generally occurs during childhood. Iron deficiency (ID) and iron deficiency anemia (IDA) are well-known extragastric manifestations in some children with *H. pylori* infection. The presence of *H. pylori* seropositivity has been observed more commonly in children with unexplained IDA than in those without IDA.\(^3,4\) Various studies showed that *H. pylori* eradication combined with iron supplementation would be more effective than iron supplementation alone for the treatment of IDA in patients with *H. pylori* infection,\(^5,6\) suggesting the causal effect of *H. pylori* infection in a substantial proportion of IDA cases in children. *H. pylori* eradication has been recommended for infected children with unexplained IDA.\(^6,7\) However, among the general population, including hundreds of children with *H. pylori* infection, studies have shown that only approximately 25% or less would develop ID or IDA.\(^8,9\) There might be different mechanisms or factors to determine whether ID or IDA will develop in children with *H. pylori* infection.

The causality of ID or IDA by *H. pylori* infection has been extensively investigated.\(^5\) Possible mechanisms of ID include acid inhibition–mediated interference with iron absorption and *H. pylori* bacterial iron utilization in the stomach.\(^10\) How the local effects of *H. pylori* in the stomach could affect systemic iron homeostasis remains unclear. Hepcidin is known as a key regulatory hormone of iron homeostasis and could cause ferroportin internalization and degradation that limit intestinal iron absorption and reduce iron transfer to the blood stream from the duodenum, macrophages, and iron-storing hepatocytes.\(^11\) Hepcidin expression is up-regulated by iron loading, inflammation, and cellular stress signals. However, hypoxia, anemia, and ID could inhibit hepcidin expression.\(^12\) Interleukin (IL) 6 is a key player activating hepcidin transcription through STAT3 phosphorylation during inflammation,\(^13\) possibly resulting in decreased total body iron. Some studies have implied that *H. pylori*–related ID might be mediated by inflammation-driven hepcidin expression,\(^14\) but others could not confirm this association.\(^15\) In addition, gastric IL-1β–associated inhibition of gastric acid secretion might be related to ID during childhood *H. pylori* infection.\(^16\) The roles of hepcidin and inflammatory cytokines in ID during *H. pylori* infection have not been substantiated. This study explored the levels of serum hepcidin and inflammatory cytokines in *H. pylori*–infected children with and without ID to clarify the role of hepcidin as a mediator or effector.

2. Materials and methods

2.1. Subjects

This study was conducted through a school-based survey regarding *H. pylori* seroprevalence administered to volunteer participants recruited by public education activities or posters at outpatient clinics at the National Taiwan University Hospital Yun-Lin branch. A total of 43 seropositive children and 43 randomly seronegative participants as control cases aged 10–18 years were enrolled in this study. Children who had clinical manifestations of acute infectious diseases were not considered for enrollment. Children who had major systemic or chronic diseases, psychological diseases, previous abdominal surgery, malnutrition, anemia with known causes, obvious causes of blood loss (e.g., gastrointestinal bleeding or menorrhagia), or had been treated for *H. pylori* infection were excluded. Written informed consent was obtained from all participants and their parents. Basic characteristics including age, gender, body weight, body height, medical history, and drug history were collected from all subjects. This study was approved by the institutional review board of the National Taiwan University Hospital.

2.2. Detection of *H. pylori* seropositivity or infection

*H. pylori* serology status was screened by detection of serum immunoglobulin (Ig) G against *H. pylori*. Sera from all participants were tested for *H. pylori* IgG antibody using an enzyme-linked immunosorbent assay (ELISA) kit (R-Biopharm AG, Darmstadt, Germany) according to the manufacturer’s instructions, with estimated sensitivity and specificity of 97% and 100%, respectively.\(^17,18\) Seropositive participants with unexplained chronic abdominal pain were recommended for clinical evaluation by a pediatric gastroenterologist regarding further upper endoscopy examinations. Gastric tissues taken during endoscopic biopsies were sent to undergo rapid urease tests, histological examination, and *H. pylori* culture. One positive result (rapid urease test, histology, or bacterial culture) for *H. pylori* was considered an active *H. pylori* infection.
2.3. Analysis of iron status, serum hepcidin, and cytokine levels

Iron profiles, including serum iron, ferritin, and total iron-binding capacity (TIBC), were examined for all subjects. The iron profiles may be affected by *H. pylori*—induced inflammation independent of true body iron status. Iron saturation was defined as serum iron (μg/dL) divided by TIBC (μg/dL). *H. pylori*—mediated ID is characterized by decreased serum iron concentration and iron saturation, but not always by decreased serum ferritin concentration. Increased serum ferritin levels with chronic inflammation are considered to reflect the process of iron sequestration in the reticuloendothelial system. Therefore, children with iron saturation less than 15% were considered to have ID in this study. Serum levels of hepcidin-25 and three cytokines (IL-1β, IL-6, and IL-8) were measured in all subjects using commercial ELISA kits (QuantiKine HS; R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. A serum hepcidin level ≤6.895 ng/mL has been reported to be associated with *H. pylori*—mediated ID in children. In this study, *H. pylori* seropositive children with a serum hepcidin level ≤6.9 ng/mL were defined as the low-hepcidin group; the others were defined as the high-hepcidin group. Iron profiles, serum hepcidin, and cytokine levels of children with and without *H. pylori* seropositivity were compared and analyzed.

2.4. Statistics

Statistical analysis was performed using STATA statistical software (v. 10; STATA Inc., College Station, TX). Numerical variables are presented as median (range) for non-normal distribution. Differences between means and medians were tested using the Wilcoxon rank-sum test. For non-Gaussian data, differences were compared using the nonparametric Mann–Whitney U test. Categorical variables are shown as number and frequency. Associations of categorical variables were tested using Fisher’s exact test or Pearson’s χ² test. Two-tailed P < 0.05 indicated statistical significance.

3. Results

3.1. Demographic and biochemical data of children seropositive and seronegative for *H. pylori*

A total of 86 children with an average age of 14.0 years were enrolled in this study; 31 children (36.0%) were boys. Among seropositive participants, 10 children (6 with ID) with chronic abdominal pain were recommended to undergo upper endoscopy examination and 9 children (6 with ID) were confirmed to have active *H. pylori*. All 9 children received anti-*H. pylori* triple therapy for 14 days and exhibited successful *H. pylori* eradication.

Table 1  Comparison of demographic and blood biochemistry data between children positive and negative for anti-*Helicobacter pylori* antibody.

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Positive <em>H. pylori</em> (n = 43)</th>
<th>Negative <em>H. pylori</em> (n = 43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>16 (37.2)</td>
<td>16 (37.2)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
<td>14.1 (13.1–15.6)</td>
<td>13.5 (13.2–13.8)</td>
<td>0.0844</td>
</tr>
<tr>
<td>Body weight (kg), median (IQR)</td>
<td>51.0 (44.4–62.4)</td>
<td>48.0 (45.0–57.0)</td>
<td>0.6101</td>
</tr>
<tr>
<td>Body height (cm), median (IQR)</td>
<td>161.0 (155.0–165.0)</td>
<td>161.0 (155.0–165.0)</td>
<td>0.8458</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>19.8 (17.9–22.0)</td>
<td>19.3 (17.4–21.5)</td>
<td>0.7007</td>
</tr>
<tr>
<td>Biochemistry data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL), median (IQR)</td>
<td>4.9 (4.7–5.1)</td>
<td>4.8 (4.6–5.0)</td>
<td>0.1933</td>
</tr>
<tr>
<td>Iron (μg/dL), median (IQR)</td>
<td>104 (75–153)</td>
<td>103 (75–162)</td>
<td>0.5804</td>
</tr>
<tr>
<td>Ferritin (ng/mL), median (IQR)</td>
<td>58.7 (13.1–96.2)</td>
<td>69.1 (44.3–107.9)</td>
<td>0.3377</td>
</tr>
<tr>
<td>TIBC (μg/dL), median (IQR)</td>
<td>371 (343–418)</td>
<td>364 (339–419)</td>
<td>0.5483</td>
</tr>
<tr>
<td>Iron saturation (%), median (IQR)</td>
<td>25.9 (19.3–38.8)</td>
<td>27.8 (22.1–39.6)</td>
<td>0.4319</td>
</tr>
<tr>
<td>Iron saturation &lt;15%, n (%)</td>
<td>8 (18.6)</td>
<td>4 (9.3)</td>
<td>0.213</td>
</tr>
<tr>
<td>Hepcidin (ng/mL), median (IQR)</td>
<td>6.5 (2.0–10.4)</td>
<td>8.6 (4.4–13.1)</td>
<td>0.1318</td>
</tr>
</tbody>
</table>

Iron saturation represents serum iron/TIBC. BMI, body mass index; IQR, interquartile range; TIBC, total iron-binding capacity.
(P = 0.1835 and P = 0.6943, respectively); however, children with seropositivity for Helicobacter pylori had significantly lower serum IL-8 levels than those with seronegativity (P < 0.0001). Interestingly, regarding the distribution of serum IL-1β and IL-6, we found that children with seropositivity could be divided into two populations with different cytokine levels (Fig. 1A, left and middle), suggesting a nonhomogeneous population.

### 3.3. Comparison of iron profiles and serum cytokine levels of H. pylori seropositive children with low and high hepcidin levels

Using a cut-off value of serum hepcidin of 6.8 pg/mL, the iron profile and cytokine levels of H. pylori seropositive children were compared (Table 2). Children with low hepcidin had significant ID patterns compared to those with high hepcidin, including lower iron, ferritin, and iron saturation and higher TIBC (P = 0.0123, 0.0001, 0.0004, and 0.0002, respectively). The prevalence of ID was also significantly higher in children with low hepcidin (33.3% versus 4.5%; P = 0.0230). These results suggested that the occurrence of H. pylori–related ID might result in reduced hepcidin levels in low-inflammatory conditions. Furthermore, serum levels of IL-1β and IL-6 were significantly lower in children with low hepcidin than in those with high hepcidin (P = 0.0151 and P = 0.0015, respectively), and the serum level of IL-8 was comparable between groups (P = 0.6443).

We examined the distribution of cytokine levels between children with low and high hepcidin levels and found that IL-1β and IL-6 levels were reduced in children with low hepcidin.

### Table 2 Comparison of iron profiles and serum cytokine levels between seropositive children with low and high hepcidin levels (hepcidin cut-off level 6.9 pg/mL).

<table>
<thead>
<tr>
<th></th>
<th>Low hepcidin (n = 22)</th>
<th>High hepcidin (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (μg/dL), median (IQR)</td>
<td>92.5 (54–104)</td>
<td>125 (93–159)</td>
<td>0.0123</td>
</tr>
<tr>
<td>Ferritin (ng/mL), median (IQR)</td>
<td>33.0 (27.0–51.8)</td>
<td>86.4 (68.3–126.7)</td>
<td>0.0001</td>
</tr>
<tr>
<td>TIBC (μg/dL), median (IQR)</td>
<td>403.5 (381–465)</td>
<td>345 (329–360)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Iron saturation (%), median (IQR)</td>
<td>20.7 (13.9–25.9)</td>
<td>36.2 (27.4–42.9)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Iron saturation &lt;15%, n (%)</td>
<td>7 (33.3)</td>
<td>1 (4.5)</td>
<td>0.023</td>
</tr>
<tr>
<td>IL-1β (pg/mL), median (IQR)</td>
<td>1.6 (0.4–8.7)</td>
<td>8.0 (7.9–9.1)</td>
<td>0.0151</td>
</tr>
<tr>
<td>IL-6 (pg/mL), median (IQR)</td>
<td>1.2 (0.5–4.8)</td>
<td>5.0 (4.9–5.4)</td>
<td>0.0015</td>
</tr>
<tr>
<td>IL-8 (pg/mL), median (IQR)</td>
<td>12.1 (9.5–18.7)</td>
<td>11.7 (11.0–23.8)</td>
<td>0.6443</td>
</tr>
</tbody>
</table>

Iron saturation represents serum iron/TIBC. IL, interleukin; IQR, interquartile range; TIBC, total iron-binding capacity.
hepcidin (Fig. 1B). The distribution patterns of IL-1β and IL-6 were similar between seronegative children and seropositive children with high hepcidin. Therefore, seropositive children with low hepcidin might be a unique population with a tendency to have an ID phenotype and low IL-1β and IL-6 serum levels.

### 3.4. Relationship of serum hepcidin, cytokine levels, and ID among *H. pylori* seropositive children

Among 43 *H. pylori* seropositive children, eight had ID. The median levels and interquartile ranges of ferritin, hepcidin, and inflammatory cytokines (IL-1β, IL-6, and IL-8) were compared between children with and those without ID (Table 3). Ferritin and hepcidin levels were significantly lower in children with ID than in the remaining 35 seropositive children without ID ($P = 0.0177$ and $P = 0.0493$). Trends of decreased serum levels of IL-1β and IL-6 were found, but there was a lack of statistical significance because of the extremely limited number of ID cases ($P = 0.0919$ and $P = 0.1045$, respectively). Using iron saturation as the severity indicator of ID, we found that serum IL-1β and IL-6 levels were significantly correlated with iron saturation among *H. pylori* seropositive children ($P = 0.0058$ and $P = 0.0127$, respectively) (Fig. 2).

### 4. Discussion

The role of hepcidin during the development of *H. pylori*-related ID in children has been widely discussed but remains controversial. H. pylori—related ID is characterized by decreased serum iron concentrations and low iron saturations, but not always decreased serum ferritin concentrations. Increased serum ferritin levels in chronic inflammation are considered to reflect the process of iron sequestration in the reticuloendothelial system. In our study, *H. pylori* seropositive children with low-serum hepcidin but a high prevalence of ID inferred that hepcidin might not be the main initiator of ID development and might be the result of feedback regulation.

During the early phase of *H. pylori* infection, IL-1β is up-regulated in the gastric antrum and corpus, acts directly on gastric parietal cells to inhibit gastric acid secretion, and regulates the transcription of several pro-inflammatory cytokines, including IL-6, IL-8, TNF, and interferon-α. In a Mongolian gerbil model of *H. pylori* infection, Takashima et al. showed elevated gastric IL-1β mRNA expression and gastric acid inhibition, which might explain one possible mechanism of acid inhibition related to ID during *H. pylori* infection. Recent studies also found increased gastric mucosa IL-1β concentrations in children infected with *H. pylori*; this was inversely associated with blood ferritin levels. One study with a small number of cases found increased serum IL-1β levels in *H. pylori*-positive children compared with *H. pylori*-negative children (median, 11.1 pg/mL versus 2.5 pg/mL); however, serum IL-6 and IL-8 levels were not different. Another study investigating patients with *H. pylori*-positive and *H. pylori*-negative gastritis or ulcers found that IL-1β was elevated in the stomach but not in

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Comparisons of ferritin, hepcidin, and serum cytokine levels between <em>H. pylori</em> seropositive children with and without ID.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With ID (n = 8)</td>
</tr>
<tr>
<td>Ferritin (ng/mL), median (IQR)</td>
<td>30.9 (28.9–34.9)</td>
</tr>
<tr>
<td>Hemicidin (ng/mL), median (IQR)</td>
<td>2.4 (0.9–5.6)</td>
</tr>
<tr>
<td>IL-1β (pg/mL), median (IQR)</td>
<td>0.9 (0.3–5.9)</td>
</tr>
<tr>
<td>IL-6 (pg/mL), median (IQR)</td>
<td>1.2 (0.6–3.6)</td>
</tr>
<tr>
<td>IL-8 (pg/mL), median (IQR)</td>
<td>13.7 (10.4–32.3)</td>
</tr>
</tbody>
</table>

ID, iron deficiency; IL, interleukin; IQR, interquartile range.
the serum of *H. pylori*-positive patients. We noticed a trend of increased serum IL-1β in *H. pylori* seropositive children compared to seronegative children, but this was not statistically significant (Fig. 1A, left). Among *H. pylori* seropositive children, serum IL-1β and IL-6 levels were significantly higher in children with high hepcidin than in those with low hepcidin (Fig. 1B, right and middle). This is consistent with a previous theory that *H. pylori*-induced IL-1β expression might trigger IL-6 production and subsequent hepcidin expression. Interestingly, the significantly high prevalence of ID in seropositive participants with low hepcidin (33.3% versus 4.5%) could not be explained by the regulation of IL-1β, IL-6, and hepcidin. It supported the hypothesis that low hepcidin might be the result of feedback regulation due to ID, and other regulatory mechanisms that trigger *H. pylori*-induced ID might exist. IL-1β might not be the only initiator of hepcidin expression.

*H. pylori*-induced inflammatory cytokine production might be different between patients and could differ between children and adults. Further studies are warranted to clarify the role of inflammatory cytokines in the development of *H. pylori*-related ID in children.

Several limitations of this study need to be considered. This study involved participants with seroprevalence, and some of them may not have an active *H. pylori* infection. This may cause less significant differences between seropositive and seronegative groups. The small number of cases restricted the power of analysis, and we could not perform a subgroup analysis because of the limited number of children with ID. The significance of cytokine changes may not be clarified because of the lack of a comparison of levels before and after bacterial eradication. Serum cytokine levels may not reflect the local expression of cytokines in the stomach. However, with these limitations, this study found that *H. pylori* seropositive children with low hepcidin levels were more likely to have a higher ID risk and lower expression of inflammatory cytokines IL-1β and IL-6. *H. pylori*-induced ID might be caused by other mechanisms, such as hypochlorhydria; however, this needs to be verified. Low hepcidin might be a feedback inhibition to maintain iron homeostasis rather than the cause of ID. More studies are warranted to determine whether the associations are clinically significant and valid. Furthermore, the detailed mechanism underlying this association requires additional investigation.

5. Conclusion

This study showed that inflammatory cytokines IL-1β and IL-6, but not IL-8, might be associated with an increased risk of ID among *H. pylori*-seropositive children.

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Conflict of interests

The authors have no conflicts of interest relevant to this article.

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Hepcidin and ID in H. pylori-infected children


