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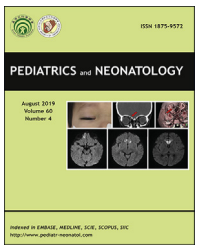

Original Article

The clinical value of plasma hepcidin levels in predicting bacterial infections in febrile children

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Key Words
bacterial infection;
children;
CRP;
hepcidin

Background: Febrile children are often evaluated for the risk of bacterial infections in the pediatric emergency department (PER). Hepcidin is an acute phase inflammatory protein. In this study, we examined the plasma hepcidin levels in febrile children.
Methods: This study was conducted at a pediatric emergency department with 123 febrile children. We measured plasma hepcidin levels using an enzyme-linked immunosorbent assay. We further evaluated clinical characteristics and routine blood tests along with the hepcidin levels.
Results: We observed significantly higher plasma hepcidin levels in bacterial enteritis ($p = 0.026$) and combined with urinary tract infection ($p = 0.007$). Furthermore, hepcidin levels had a significantly positive correlation with CRP level and length of hospital stay ($R = 0.296$, $p = 0.001$ and $R = 0.213$, $p = 0.018$). Hepcidin levels greater than 65 ng/mL also more accurately predicted bacterial infections than values below 65 ng/mL (11.7% vs. 2.1%, Odds ratio 8.4, 95% confident interval 1.7—40.9, $p = 0.002$).
Conclusion: This study provides evidence that febrile children with bacterial infection have higher plasma hepcidin levels, and the values correlated with CRP level and length of hospital stay. Therefore, hepcidin values can potentially be adopted as a biomarker for identifying febrile children with bacterial infection, particularly bacterial enteritis and urinary tract infection.

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1. Introduction

Fever is among the most common causes for which children seek medical care.[1] In most cases, the origin of a child's fever is self-limited viral infection; however, identifying a bacterial infection, which would require immediately treatment, is vital.[2] When approaching a patient, in addition to taking a history and performing a physical examination, laboratory studies have traditionally helped clinicians predict bacterial infections; such tests include complete blood count (CBC) with differential count, band to neutrophil ratio, and C-reactive protein (CRP).[3–6]While managing a fever remains a challenge in clinical conditions because patients with occult bacteremia may initially present as being well,[7] viral illnesses may induce secondary bacterial infections. Biomarkers have limitations with regard to evaluating bacterial infection at first glance in PERs, while sensitivity and specificity mean that diagnostic values may be questionable.

Hepcidin, a 25-amino-acid peptide that is primarily synthesized by hepatocytes, was discovered in 2000. This peptide plays a vital role in iron homeostasis and as an immune mediator.[8–11] Hepcidin blocks ferroportin, which exports iron, leading to decreased intestine absorption, iron storage of hepatocytes, and iron release from tissue, thus reducing the iron released into peripheral circulation.[12–15] Furthermore, hepcidin is a type II acute phase protein that provides a connection between iron metabolism and inflammation.[16–18] Interleukin-6 (IL-6), a cytokine induced by inflammation, up-regulates hepcidin in response to both viral and bacterial infections,[16,19,20] while hepcidin induction decreases iron being released from serum and tissue, which thus restricts invading pathogen growth.[16] Some studies have shown that the relationship between hepcidin and sepsis has been effectively used to determine the prognosis of critical patients.[21–23]

Although we have commonly applied traditional biomarkers to patients, no single biomarker can perfectly predict bacterial infection in febrile children, and they usually have pitfalls when a PER doctor is evaluating patient. Hepcidin can be induced by immune response to pathogen invasion and can regulate iron levels to enhance a host's defense.[10] Therefore, in this study, we aim to compare CRP with hepcidin and determine the relationship between hepcidin levels and bacterial infections in febrile children.

2. Patients and methods

2.1. Patients

This study consisted of a total of 123 febrile children that visited our PER at Kaohsiung Chang Gung Hospital in Taiwan through March, 2011 to June, 2017. The study included that pediatric febrile patients under the family's consent and did laboratory study at the same day. We excluded the patients more than 18 years old. The cases were classified into bacterial and non-bacterial infection groups based on the clinical cultural finding. The Institutional Review Board of Chang Gung Memorial Hospital approved this study (#102-5947C), and we obtained the written informed consent from the guardians of all participants. Blood samples were immediately placed in heparin-containing tubes, and the remaining aliquots of plasma were stored at −80 °C until needed for analysis.

2.2. Plasma hepcidin measurement using enzyme-linked immunoassay (ELISA)

The ELISA kits used to analyze the plasma and urine hepcidin-25 were commercially available competitive assays that used synthetic hepcidin (Catalog Number: S-1337, Bachem Biosciences, St. Helens, United Kingdom, range: 0—25 ng/mL), and the methodology and performance characteristics have been previously described in another study.[24]

2.3. Statistical analysis

All data are presented as the mean ± standard error. We analyzed quantitative data with the Student's *t*-test or one-way ANOVA when necessary, with $p < 0.05$ denoting statistical significance. All statistical tests were performed with SPSS 22.0 for Windows XP (SPSS, Inc., Chicago, Illinois).

3. Results

3.1. Patient characteristics

Of the 123 febrile children enrolled in this study, 64 patients (52%) were male, and the mean age of the patients was 2.8 ± 0.2 years old. The patients' basal characteristics are provided in Table 1.

3.2. Higher plasma hepcidin levels in bacterial infections

Based on our cultural results, we found no pathogenic bacteremia, six cases of bacterial enteritis (5 *Salmonella*

Table 1 Subjects' characteristics.

Number	Subjects	Bacterial culture		p-value
	112	11		
		Stool	Urine	
		6	5	
Mean Age (months)	35.4 ± 2.9	19.1 ± 8.5		0.084
Male gender, N (%)	59 (52.7%)	5 (45.5%)		0.650
Length of hospital stay (day)	3.7 ± 0.2	5 ± 0.5		**0.044**
CRP (mg/L)	27.0 ± 4.1	34.9 ± 9.5		0.546
White blood cells (1000/uL)	9.5 ± 0.5	8.5 ± 1.1		0.535
Hemoglobin (g/dL)	12.1 ± 0.1	11.8 ± 0.4		0.445
GOT (IU/ml)	48.4 ± 4.2	43.3 ± 6.5		0.745
GPT (IU/ml)	34.9 ± 6.9	23.8 ± 3.3		0.663
Hepcidin (ng/mL)	59.8 ± 4.0	97.6 ± 17.6		**0.007**

Bold type indicates $p < 0.05$.

and one *Campylobacter jejuni*), and five cases of urinary tract infection (4 *Escherichia coli* and one *Klebsiella pneumoniae*) in our febrile children. As demonstrated in Table 1, higher plasma hepcidin levels and longer length of hospital stay were observed in febrile children with bacterial enteritis or combined with bacterial urinary tract infection ($p = 0.026$ and 0.006; $p = 0.007$ and 0.044, respectively). However, the relationship between CRP levels and bacterial infection had no statistical significance ($p = 0.546$).

3.3. Correlation between CRP level and length of hospital stays and hepcidin levels in febrile children

In this study, we demonstrated that plasma hepcidin levels are correlated with patients' CRP levels and length of hospital stays ($R = 0.296$, $p = 0.001$ and $R = 0.213$, $p = 0.018$, respectively), as shown in Figs. 1 and 2, respectively. Moreover, patients' CRP instead of total WBC levels are also correlated with length of hospital stays. ($R = 0.296$, $p < 0.001$, and $R = 0.13$, $p = 0.159$, respectively). Furthermore, hepcidin levels greater than 65 ng/mL can more accurately predict bacterial infection than values below 65 ng/mL (18.8% vs. 2.7%, Odds ratio 6.3, $p < 0.001$).

4. Discussion

Based on our study, higher hepcidin levels were capable of indicating a bacterial infection, particularly bacterial enteritis and urinary tract infection. Furthermore, higher hepcidin levels were associated with longer hospital stays in febrile children with bacterial enteritis and a urinary tract infection. We also found that plasma hepcidin levels are correlated with patients' CRP levels. Hepcidin levels greater than 65 ng/mL were observed to more accurately predict bacterial infection than values below 65 ng/mL. However, no statistical significance was found between CRP levels and bacterial infection ($p = 0.546$).

Among the biomarkers applied to diagnose suspicious bacterial infections, CBC with white blood cell (WBC) count

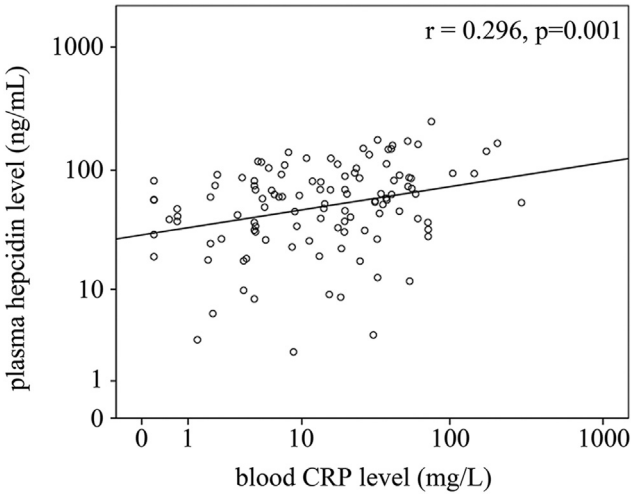

Figure 1 Plasma hepcidin level is correlated to CRP level.

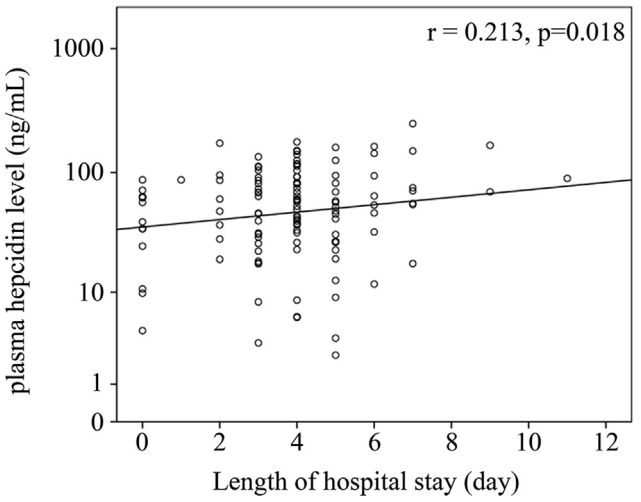

Figure 2 Plasma hepcidin level is correlated to length of hospital stay.

and CRP level are commonly used in patients.[25,26] Furthermore, leukocytosis or leucopenia may be an indicator for sepsis. However, they have low sensitivity and specificity and do not reliably predict septic shock in pediatric patients, thus limiting their use in predicting bacterial infections in children.[27] CRP is an acute phase protein that responds to various kinds of cytokine stimulation and is commonly used due to its wide availability.[28] In general, CRP is clinically applied to distinguish bacterial infection from viral or other infections, but the sensitivity and specificity have varied in studies.[29] CRP levels also increase in the inflammatory process, such as trauma, surgery, and rheumatic disease.[27] *Sanders* et al. reviewed CRP's diagnostic accuracy in febrile neonates and children and found that CRP has a moderate ability for ruling out serious bacterial infections in febrile children but cannot exclude all bacterial infections due to its poor sensitivity.[30] Because of its low specificity, CRP needs to be combined with other diagnostic tools, such as procalcitonin as a biomarker panel, in order to improve accuracy.[28] Despite the common use of CRP as a diagnostic tool to detect bacterial infections, it does not provide clinicians with the ability to make a definite diagnose of a bacterial infection.

Clinical studies have demonstrated that cytokine levels fluctuate when encountering a pathogen invasion. Some pathogen-associated-molecules, such as lipopolysaccharide (LPS) from Gram negative bacteria, stimulate the immune system and induce subsequent cytokine release.[28,31] Interleukin 6(IL-6) released by macrophages is one of the cytokine responses to bacterial infections; it not only up-regulates hepcidin expression but also enhances CRP production.[16,19,20,28] *Riedel* et al. reviewed biomarkers for sepsis and found that some studies used IL-6 to detect sepsis early in neonates and emergency room patients, but a single-center prospective study of neonates with sepsis showed that IL-6 did not have a significant positive predictive value (PPV) for the early prediction of sepsis.[29]

Hepcidin plays an important role in immune mediation, and IL-6 is among the key cytokines to up-regulate hepcidin expression[16,18–20] and also regulates iron metabolism. In contrast, the hepcidin level increases in inflammation or

infection, thus limiting the iron released into the blood and disrupting pathogen growth.[14,16] Kemna et al. have demonstrated that there were temporal associations between plasma cytokines, and hepcidin levels in 10 healthy individuals after LPS injection.[32] IL-6 was noticeably induced within 3 h after injection, while urinary hepcidin peaked within 6 h, followed by CRP with maximum levels detected 22 h after injection.[32] A prospective single center study proposed that the parameters of iron metabolism reflected serum iron availability and predicted outcomes in ICU patients.[22] In line with the study done by *Wu* et al. hepcidin is increased 4-fold in infants with sepsis.[23] Furthermore, Kossiva et al. found that serum hepcidin and ferritin values were significantly higher, whereas serum iron was significantly lower in children with infection.[33]

In our study, we discovered that plasma hepcidin levels are correlated with the length of hospital stays ($R = 0.213$, $p = 0.018$), which few studies have discussed the correlation between hepcidin and hospital stay length. Our previous study focusing on patients with Kawasaki disease also showed that plasma hepcidin levels both before and after IVIG treatment were positively correlated with patients' length of hospital stays ($R = 0.217$, $p = 0.046$, and $R = 0.381$, $p < 0.0001$, respectively).[34] In cardiac surgical patients, plasma hepcidin concentration related to outcome and preoperative anemia was shown to be associated with prolong intensive care unit stays.[35,36] Elevated hepcidin levels indicates an iron deficiency and anemia that are often associated with an increased hospital stay length.[37]

Our study has some limitations that should be mentioned. First, the correlation coefficient between hepcidin and CRP and length of hospital stay is less than 0.4, although it is statistically significant. Second, the study that the cases numbers of bacterial infection group are relative small, and it could statically underpower the correlation between CRP and bacterial infection. Third, we did not obtain sputum culture or viral culture at PER because of equipment limitation, and we could not identify the pathogen of airway infection. We still need collect more specimen cultures to clarify the role of hepcidin in other kinds of bacterial infection.

5. Conclusion

This study provided another view that hepcidin may identify bacterial infections in febrile children, particularly bacterial enteritis and urinary tract infection. Hepcidin levels also correlated to CRP levels and length of hospital stays. Plasma hepcidin levels have a promising future with regard to distinguishing bacterial and viral infections in febrile children.

Conflicts of interest

The authors hereby declare to have no conflicts of interest related to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2018.09.001.