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Analysis of Serum Hepcidin Levels in Children with Iron Deficiency

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KEYWORDS

Hepcidin, iron deficiency, anemia, children, ELISA, biomarker.

ABSTRACT:

Introduction: Iron deficiency, with or without anemia, remains one of the most prevalent nutritional deficiencies among children worldwide, including Indonesia. Hepcidin, a peptide hormone produced by the liver, serves as the principal regulator of systemic iron homeostasis. Its levels decrease during iron deficiency, rendering it a potential biomarker for early detection before anemia develops. However, limited research has examined serum hepcidin in Indonesian children with varying iron statuses.

Objective: To analyze serum hepcidin levels among children with normal iron status, stage I iron deficiency, stage II iron deficiency, and iron deficiency anemia (IDA).

Methods: A cross-sectional study was conducted from February to April 2025 involving children aged 1–18 years residing in 15 orphanages in Makassar City. Subjects were classified into four groups based on hematological and biochemical parameters (Hb, MCV, MCH, reticulocytes, and ferritin): normal, stage I ID, stage II ID, and IDA. Serum hepcidin concentrations were quantified using the Human Hepcidin ELISA kit (BT-Assay E1019Hu). Statistical analysis employed ANOVA, Kruskal–Wallis, and Mann–Whitney tests with a significance level of $p < 0.05$.

Results: A total of 117 children participated in the study (normal = 30; stage I ID = 30; stage II ID = 30; IDA = 27). Significant differences were observed across iron status groups for Hb, MCV, MCH, and ferritin ($p < 0.05$). Serum hepcidin levels declined progressively with worsening iron deficiency, reaching their lowest levels in the IDA group ($p = 0.003$). Hepcidin levels were not significantly different between normal, stage I ID, and stage II ID groups, but were markedly reduced in IDA.

Conclusion: Serum hepcidin levels significantly decrease in children with IDA, confirming its potential as a sensitive biomarker for detecting iron deficiency before anemia onset. Incorporating

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hepcidin measurement into standard iron status assessments may enhance the accuracy of early diagnosis and improve preventive interventions.

1. Introduction

Iron deficiency is the most prevalent micronutrient deficiency affecting children worldwide and a major contributor to anemia. In Indonesia, iron deficiency anemia (IDA) persists as a significant public health challenge. The 2018 National Health Survey (Riskesdas) reported that anemia affected 26.8% of children aged 5–14 years and 32% of adolescents aged 15–24 years. Worryingly, the prevalence of anemia in the pediatric population (0–19 years) has increased over the last decade. Early-stage iron deficiency, even before anemia manifests, can irreversibly impair motor, cognitive, and behavioral development if left untreated.[1]

The diagnosis of pediatric iron deficiency and IDA traditionally relies on a combination of clinical evaluation and laboratory tests. Standard iron status assessment includes hematological parameters such as hemoglobin (Hb), hematocrit, and erythrocyte indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH]), alongside biochemical markers like serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation [2,3] Classic IDA presents as microcytic hypochromic anemia with reduced Hb, MCV, MCH, and low serum ferritin.[3,4] However, these conventional markers may not always reflect the true severity of iron deficiency, particularly in its initial stages. This limitation has driven the search for more precise and specific biomarkers for early detection.[5]

Hepcidin, a 25-amino acid peptide hormone primarily synthesized by the liver, is the master regulator of systemic iron homeostasis.[6] It functions by binding to the iron export protein ferroportin, which is present on the surface of intestinal enterocytes, macrophages, and hepatocytes. This binding induces the internalization and degradation of ferroportin, thereby blocking iron entry into the circulation. Consequently, hepcidin acts as a negative regulator: high hepcidin levels reduce dietary iron absorption and inhibit the release of stored iron, whereas low hepcidin levels facilitate iron absorption and mobilization.[6–8]

In states of absolute iron deficiency, the suppression of hepcidin synthesis is a critical physiological adaptation to increase iron availability. Research suggests that serum hepcidin is a highly sensitive indicator that responds early in the course of iron deficiency, often before detectable declines in Hb, ferritin, or transferrin saturation.[5,9] This characteristic positions hepcidin as a promising biomarker for identifying early-stage iron deficiency.

While numerous international studies have explored hepcidin levels in IDA, data from the pediatric population in Indonesia remain scarce.[10,11]

2. Objectives

This study was therefore conducted to evaluate serum hepcidin levels across different stages of iron status—normal, stage I ID, stage II ID, and IDA—in Indonesian children. The findings are expected to provide scientific evidence supporting the role of hepcidin in the early diagnosis of iron deficiency and to inform the development of improved diagnostic and preventative strategies for IDA in this population.

3. Methods

Study Design and Participants

This cross-sectional laboratory study was conducted from February to April 2025 in Makassar, Indonesia. The study population comprised children aged 1 to 18 years residing in 15 randomly selected orphanages (*Lembaga Kesejahteraan Sosial Anak*, LKSA). Participants who met the operational criteria for normal iron status or iron deficiency (stage I, II, or IDA) following initial screening and whose parents or legal guardians provided informed consent were included in the hepcidin analysis. Exclusion criteria were: age under 1 year, presence of acute infection, immunocompromised status (e.g., long-term corticosteroid use, malignancy, HIV infection), recent iron therapy (oral supplementation or transfusion), severe malnutrition (wasting), and obesity.

Based on screening laboratory results, subjects were classified into four groups: normal iron status, stage I ID, stage II ID, and IDA. Stage I ID was defined by serum

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ferritin <30 ng/mL with normal Hb, MCV, and MCH. Stage II ID was defined by ferritin <30 ng/mL with decreased MCV and/or MCH but normal Hb. IDA was defined by ferritin <30 ng/mL with anemia (low Hb) and low MCV and MCH. The study protocol was approved by the Biomedical Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar.

Data Collection

Demographic data (name, age, gender) and anthropometric measurements for nutritional status assessment were recorded for all participants. Venous blood (3 mL) was collected for laboratory analysis. The initial screening included a complete blood count (Hb, MCV, MCH), reticulocyte count, and serum ferritin analysis to classify subjects into the appropriate iron status groups. Serum was separated via centrifugation and stored at -20°C until analysis. Subsequently, serum hepcidin levels were measured using a Human Hepcidin ELISA Kit (BT-Assay E1019Hu) on a Thermo Scientific ELISA reader at the Hasanuddin University Medical Research Center, Makassar, Indonesia.

Data Analysis

Data were analyzed using SPSS version 24. Subject characteristics were summarized using frequencies, means, standard deviations (SD), medians, and ranges. A p-value < 0.05 was considered statistically significant. The Kolmogorov-Smirnov test was used to assess data normality. The Chi-square test was used for comparisons of categorical variables (gender, nutritional status). The Kruskal-Wallis test was employed for non-normally distributed numerical data across the four groups, followed by the Mann-Whitney U test for pairwise comparisons. ANOVA was used for normally distributed data.

4. Results

A total of 117 children met the inclusion criteria and were enrolled in the study: 30 with normal iron status, 30 with stage I ID, 30 with stage II ID, and 27 with IDA (**Figure 1**).

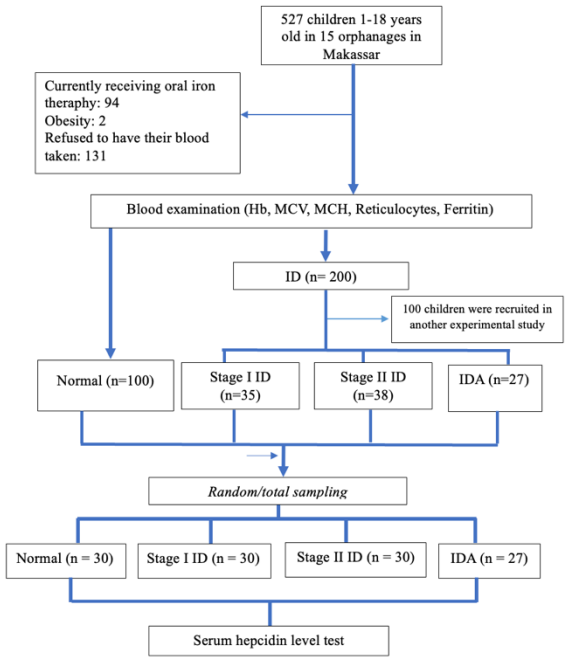

Figure 1. Flowchart of participant selection and classification based on iron status for serum hepcidin analysis.

Table 1. Demographic and Nutritional Characteristics of Study Participants by Iron Status

Variables	Normal (n = 30)	Stage I ID (n = 30)	Stage II ID (n = 30)	IDA (n = 27)	p-value
Sex					0.142*
Male	20 (66.7%)	12 (40.0%)	15 (50.0%)	11 (40.7%)	
Female	10 (33.3%)	18 (60.0%)	15 (50.0%)	16 (59.3%)	
Age Group (years)					0.072*
1–6	7 (23.3%)	1 (3.3%)	3 (10.0%)	6 (22.2%)	
>6–12	10 (33.3%)	13 (43.3%)	8 (26.7%)	13 (48.1%)	
>12–18	13 (43.3%)	16 (53.3%)	19 (63.3%)	8 (29.6%)	
Nutritional Status					0.877*

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Variables	Normal (n = 30)	Stage I ID (n = 30)	Stage II ID (n = 30)	IDA (n = 27)	p-value
Wasted	3 (10.0%)	4 (13.3%)	3 (10.0%)	3 (11.1%)	
Normal	22 (73.3%)	18 (60.0%)	18 (60.0%)	19 (70.4%)	
Overweight	5 (16.7%)	8 (26.7%)	9 (30.0%)	5 (18.5%)	

Note: * Chi-square test.

The demographic and nutritional characteristics of the participants are presented in **Table 1**. The mean age of participants was comparable across the four groups, and there was a nearly equal distribution of males (49.6%) and females (50.4%). No significant differences in age, gender, or nutritional status were observed among the groups, indicating that the study groups were homogenous.

Table 2. Laboratory Parameters of Study Participants by Iron Status

Variables	Normal (n = 30)	Stage I ID (n = 30)	Stage II ID (n = 30)	IDA (n = 27)	p-value
Hemoglobin (g/dL), mean	13.5	13.4	12.7	10.5	<0.001**
MCV (fL), median (min–max)	83.9 (80.3–89.4)	82.1 (80.0–88.7)	76.3 (68.2–79.5)	71.7 (52.5–78.5)	<0.001*
MCH (pg), median (min–max)	28.1 (27.0–33.1)	27.8 (27.0–30.1)	25.2 (21.3–26.6)	23.6 (15.5–26.9)	<0.001*
Reticulocytes (%), mean	0.84	1.03	0.88	0.92	0.103**
Ferritin (ng/mL), median (min–max)	53.1 (30.9–98.0)	12.9 (5.1–29.3)	12.0 (2.05–28.4)	12.4 (2.08–29.7)	<0.001*
Hepcidin (ng/mL), median (min–max)	289.5 (22.0–1881.7)	321.5 (12.9–1216.6)	212.4 (2.4–798.7)	48.3 (1.12–716.2)	0.003*

Note: * Kruskal–Wallis test; ** ANOVA test.

Laboratory parameters related to iron status are summarized in **Table 2**. There were highly significant differences in Hb, MCV, MCH, and ferritin levels across the four groups ($p < 0.001$ for all). A clear downward trend was observed from the normal group to the IDA group for these parameters. In contrast, reticulocyte counts did not differ significantly among the groups ($p = 0.103$).

Serum hepcidin levels also showed a statistically significant difference across the groups ($p = 0.003$). The median hepcidin level was highest in the stage I ID group, followed by the normal and stage II ID groups, and was drastically lower in the IDA group.

Table 3. Comparison of Serum Hepcidin Levels Between Groups

Comparison Group 1	Comparison Group 2	Median Hepcidin (ng/mL) – Group 1	Median Hepcidin (ng/mL) – Group 2	p-value
Normal	Stage I ID	289.5	321.5	0.859
Normal	Stage II ID	289.5	212.4	0.344
Normal	IDA	289.5	48.3	**<0.001**
Stage I ID	Stage II ID	321.5	212.4	0.255
Stage I ID	IDA	321.5	48.3	**<0.001**
Stage II ID	IDA	212.4	48.3	**0.014**

Note: Mann–Whitney U test. Significant p-values (<0.05) are shown in bold.

Post-hoc pairwise comparisons using the Mann-Whitney U test were performed to identify specific inter-group differences in hepcidin levels (**Table 3**). The analysis revealed that serum hepcidin levels in the IDA group were significantly lower than those in the normal group ($p < 0.001$), the stage I ID group ($p < 0.001$), and

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the stage II ID group (p = 0.014). However, there were no statistically significant differences in hepcidin levels between the normal, stage I ID, and stage II ID groups. These results indicate that a significant drop in hepcidin occurs predominantly when iron deficiency progresses to overt anemia.

5. Discussion

This study investigated the utility of serum hepcidin as a biomarker for iron deficiency in a sample of Indonesian children. The key finding is that serum hepcidin levels were significantly suppressed only in children with established iron deficiency anemia (IDA), but not in those with earlier, non-anemic stages of iron deficiency.

Consistent with established pathophysiology, conventional iron status markers such as Hb, MCV, MCH, and ferritin progressively declined as iron status worsened, with the lowest values observed in the IDA group. This aligns with previous research by Andriastuti et al. (2020)[1], Kumar et al. (2023)[2], and Motulo et al. (2023) [3]. The lack of a significant difference in ferritin between the stage I, II, and IDA groups may be attributed to its nature as an acute-phase reactant. Inflammation or subclinical infections can elevate ferritin levels independently of iron stores, potentially masking the true extent of deficiency until it becomes severe.[4,5] The reticulocyte count did not vary significantly across the groups, which is consistent with findings that suggest it is an unreliable marker for differentiating stages of iron deficiency, as bone marrow compensation can maintain reticulocyte production within a normal range until anemia is severe.[6,7]

The central finding of our study is the behavior of hepcidin across the iron deficiency spectrum. The profound reduction of hepcidin in the IDA group confirms its physiological role in maximizing iron availability during severe iron depletion and is consistent with studies by Choi et al. (2012),[9] who also reported the lowest hepcidin levels in anemic children. This supports the utility of hepcidin measurement as a strong indicator of IDA.

Interestingly, we did not observe a statistically significant decrease in hepcidin levels in the stage I and stage II ID groups compared to the normal group. This finding, also noted by Pasalina et al. (2025),[11] suggests that the physiological signal for hepcidin suppression may not be sufficiently potent during the early stages of iron store depletion, before systemic iron deficiency impacts erythropoiesis. Several factors could contribute to this observation. First, hepcidin is an acute-phase protein, and its levels can be influenced by subclinical inflammation, hormonal fluctuations, and other factors unrelated to iron status, even in apparently healthy children.[10] Second, genetic variability, particularly in regulatory genes like TMPRSS6, can alter hepcidin response to iron signals.[12,13] Finally, the wide inter-individual variation in hepcidin levels, as evidenced by the large ranges observed in our data, may have masked a subtle downward trend in the non-anemic groups, necessitating a larger sample size to detect a significant difference.

Despite its limitations in detecting early-stage ID in this study, the measurement of hepcidin holds significant clinical potential. Its ability to distinguish IDA from anemia of chronic disease (ACD) remains a key advantage, as hepcidin is typically low in IDA but elevated in ACD.[14–18] Furthermore, a very low hepcidin level can serve as a confirmatory marker for IDA, guiding prompt initiation of iron supplementation to mitigate the neurodevelopmental consequences of iron deficiency.

This study's strength lies in its novel analysis of hepcidin across four distinct stages of iron status in an Indonesian pediatric cohort from a homogenous community setting, which minimizes dietary variability. However, the study has limitations. Its cross-sectional design precludes causal inference and the tracking of hepcidin levels over time. Furthermore, the absence of inflammatory markers (e.g., C-reactive protein, IL-6) prevented us from controlling for the potential confounding effect of subclinical inflammation on hepcidin levels. Future longitudinal studies with larger sample sizes are warranted to establish pediatric cut-off values for hepcidin and to elucidate its correlation with other iron parameters over time.

6. Conclusion

In this study of Indonesian children, serum hepcidin levels were significantly lower in those with iron deficiency anemia compared to children with normal iron status or non-anemic iron deficiency. This finding confirms that profound hepcidin suppression is a

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hallmark of advanced iron depletion. While hepcidin did not emerge as a sensitive marker for the early stages of iron deficiency in this cohort, its measurement remains a valuable tool for confirming the diagnosis of IDA and holds promise for differentiating it from other types of anemia.

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