ORIGINAL ARTICLE

Hepcidin Levels in Response to Oral Iron Therapy in Children with Anemia

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ABSTRACT

Objective: To estimate the change in serum hepcidin levels and its correlation with change in hemoglobin (Hb) level during the initial two weeks of oral iron therapy in children with iron deficiency anemia (IDA).

Methods: A prospective observational study was carried out in children aged 2-12 years with IDA. Children with severe anemia (Hb < 7 g/ dL), those with fever, infections, history of oral iron intake or blood transfusion within the preceding three months, or intolerant to oral iron were excluded. Serum hepcidin-25 was assessed using ELISA-based kits on day 0 (pre-therapy), after 24 hours and 14 days of starting oral iron therapy.

Results: Out of 78 children who were screened for inclusion, we included 64 children with IDA with a mean (SD) hemoglobin of 8.81 (1.22) g/dL. The baseline mean (SD) serum hepcidin-25 levels [7.81 (4.88) ng/mL] increased significantly to 8.38 (4.96) at 24 hours and 9.51 (5.2) on day 14 of oral iron therapy ($P < 0.001$). 63 children showed a good response to oral iron therapy. No significant correlation was observed between baseline hepcidin levels with change in hemoglobin on day 1 (r = -0.10, $P = 0.40$) or day 14 (r = -0.10, $P = 0.43$) of therapy.

Conclusion: Serum hepcidin levels rise significantly as early as 24 hours after starting oral iron therapy and should be explored to assess response to oral iron therapy in children with anemia.

Keywords: *Biomarker, Iron deficiency anemia, Response*

INTRODUCTION

One of the earliest parameters used to assess response to oral iron therapy in patients with iron deficiency anemia (IDA) is a rise in the reticulocyte count around the 5-7th day of therapy. A rise in hemoglobin level of ≥ 1 g/dL after two weeks of therapy is also used to define response to oral iron therapy [1]. Both parameters entail a substantial amount of time to ascertain response to therapy. This emphasizes the need for a biomarker for predicting early response to iron therapy to guide treatment.

Hepcidin is the main iron recycling hormone as it acts on ferroportin which transports iron extracellularly to the plasma, while down regulating absorption and release of iron after binding to hepcidin and forming hepcidin-ferroportin complex, which is internalized and degraded in states of adequate or high iron stores [2]. Hepcidin levels decrease in iron deficiency to facilitate maximal iron absorption [3]. A few studies have shown that hepcidin levels rise promptly in response to intravenous and oral iron therapy in patients suffering from IDA [4,5]. In a study carried out in adults, it was seen that hepcidin levels start rising as early as within an hour of oral iron therapy with a peak rise occurring at 24 hours [4]. However, there is paucity of such studies in children. We conducted a prospective observational study to evaluate the serum hepcidin levels following oral iron therapy in children of IDA aged 2-12 years.

METHODS

The study was conducted in the Departments of Pediatrics and Biochemistry in Atal Bihari Vajpayee Institute of Medical Science and Dr Ram Manohar Lohia Hospital, New Delhi. A prior approval from the institutional

ethics committee was taken. A written informed consent was obtained from the parents/guardians and a verbal assent was obtained from the participants as needed.

Children aged 2-12 years, recently diagnosed with IDA, were consecutively recruited from the pediatric outpatient department between November 01, 2019 and March 31, 2021. IDA was diagnosed and its severity was categorized as mild, moderate or severe, based on age-dependent criteria [6,7]. For children aged 2-5 years, Hb < 11 g/dL and serum ferritin < 12 ng/mL and for those aged 5-12 years, Hb < 11.5 g/dL and serum ferritin <15 ng/mL were considered diagnostic of IDA. The cut-off for transferrin saturation was taken as < 16% for diagnosing IDA in all ages. Children with severe IDA (Hb < 7 g/dL), those suffering from fever or acute infective illness, having a history of oral iron intake or blood transfusion within the preceding three months, or with a history of intolerance to oral iron were excluded.

At the time of recruitment, a detailed history and physical examination including anthropometric assessment were recorded. Anthropometry was interpreted using Indian Academy of Pediatrics (IAP) growth charts [8]. A venous blood sample was drawn at baseline (D0) for the estimation of a complete blood count (CBC) including red blood cell (RBC) indices, serum iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), transferrin saturation, serum ferritin, serum hepcidin and C-reactive protein (CRP). Oral iron therapy (syrup ferrous sulphate, 6 mg elemental iron per mL) was administered in a dose of 3 mg/kg/day of elemental iron. The first dose of oral iron was administered on day 0 as a single morning dose (3 mg/kg) under the direct supervision of the investigator. The caregivers/parents were advised to administer the subsequent daily doses of iron (3 mg/kg/day of elemental iron) in two divided doses. All children were followed up after 24 hours of starting oral iron therapy (D1) wherein sample was drawn for repeat assessment of serum hepcidin and CBC, and on day 14 (D14) when venous blood samples were drawn for the estimation of CBC, serum iron, TIBC, UIBC, transferrin saturation, serum ferritin, CRP, and serum hepcidin assessment. Serum transferrin, serum iron, TIBC and UIBC were analyzed as recommended by Iron Panel of the International Council for Standardization in Hematology (ICSH) [9]. Venous blood (2 mL) was collected in a plain vial and sera separated and stored at −20°C for measuring serum hepcidin using complement ELISA technique (DRG hepcidin 25 (bioactive) HS ELISA kit; Marburg, Germany). Quantitative estimation of CRP was done on serum samples by microsliding method on day 0 and day 14 (VITROS Orthos CRP slides) and CRP > 1 mg/dL was considered as positive. Any child found to have a positive C-reactive protein (> 1 mg/dL) at initial assessment was excluded from the study. Compliance and side effects of iron therapy such as gastritis, constipation, nausea and metallic taste were enquired telephonically on day 4, 8 and 11. Caregivers were also asked to maintain a diary for recording the compliance and side effects of therapy, if any. Good response to oral iron was defined as rise in Hb ≥ 1 g/dL after 2 weeks of daily therapy [1].

Sample size calculation was based on a study by Choi et al [10] wherein baseline serum hepcidin level of 2.01 (2.30) ng/ml were reported in children with IDA and also on the study by Moretti et al [4] who demonstrated that serum hepcidin levels increased by a factor of 2.7 after 24 hours of oral iron administration in adult females. Taking these values as reference and expecting a rise in hepcidin levels by 1.5 times after 24 hours of oral iron therapy, a sample of 64 children with IDA was needed considering the power of study as 90%, level of significance as 5%, and a possible loss to follow up of 20%.

Statistical analysis: Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software, version 21.0. Categorical variables were presented as numbers (*n*) and percentages and continuous variables were presented as the mean (standard deviation, SD), in case of normal distribution, or as median (interquartile range) in case of non-normal distribution. The comparison of quantitative variables was done using independent t test. Paired t test was used for comparing the change in hemoglobin and hepcidin levels at different time points (D0, D1 and D14) during follow up. Pearson correlation coefficient was used for correlation between baseline hepcidin and change in hemoglobin and iron profile. *P* value < 0.05 was considered as statistically significant.

RESULTS

Out of 78 children who were screened for inclusion in this study, 69 children were eligible for inclusion. **Fig. 1** depicts the flow of participants in this study. 64 children aged 2-12 years (30 boys, 34 girls), with mean (SD) age of 7.39 (3) years diagnosed with IDA were followed up and included in the final analysis. 50 (78.13%) children were aged ≥ 5 years while 14 (21.87%) were aged 2-5 years. Nutritional evaluation of the study participants revealed that 6/64 (9.38%) had weight-for-age less than 3rd centile and 7/64 (10.94%) had height-for-age less than 3rd centile as per IAP growth charts. The most common symptoms present in children suffering from IDA were decreased appetite (*n* = 21, 32.8 %) followed by vomiting (*n* = 13, 20.3%), pain abdomen (*n* = 10, 15.6%), and fatigue (*n* = 9, 14.1%). The mean (SD) CRP levels (mg/dL) on day 0 and day 14 were 0.6 (0.22) and 0.5 (0.28), respectively (**Table I**). None of the children reported any side effects severe enough to discontinue oral iron. Two of the 64 children (3.13%) developed constipation which responded to lifestyle modification and laxatives.

Pre-therapy (D0) and day 14 hematological parameters have been depicted in **Table I**. Almost all the patients showed a good response to oral iron therapy on day 14 with only one child being a non-responder. The mean (SD) baseline serum hepcidin levels (ng/mL) were 3.24 (0.91) in children <5 years and 9.09 (4.77) in children ≥ 5 years. A significant rise in serum hepcidin levels was observed in the whole cohort on days 1 and 14 following oral iron therapy (both P < 0.001). The levels rose significantly within the two age sub-groups as well. **Fig. 2** shows the hepcidin values at different time points in the study population in the two age groups. Hepcidin levels were not statistically significant between males and females at any point of time. No significant correlation was observed between baseline hepcidin levels with change in hemoglobin, RBC count, MCV, MCH, MCHC and serum ferritin after 24 hours and 14 days of therapy (**Table II**).

DISCUSSION

The present study was carried out in 64 children aged 2-12 years with IDA to assess the changes in hepcidin levels after starting oral iron therapy. It was observed that pre-therapy mean serum hepcidin levels were 7.81 (4.88) in all children with IDA with lower values seen in children < 5 years compared to older children. A significant rise in serum hepcidin levels was observed in all the children after starting oral iron therapy on day 1 (107%) and day 14 (122%). Although, we did not find any correlation between pre-therapy hepcidin levels and change in hemoglobin levels after 1 and 14 days of treatment.

The main limitation of this study was the absence of a control group, given that lack of age and population appropriate normative values. The challenges associated with using hepcidin as a monitoring tool

in IDA include its poor availability and cost. Further, it has poor specificity in infections, inflammation and even in obesity [11]. The strength of this study is the that CRP assessment was done alongside hepcidin assays on day 0 and 14, and only children who had CRP levels < 1 mg/dL (non-reactive) were included, thereby exclusing children with underlying inflammation or infection.

The pre-therapy levels observed in the present study were lower in comparison to those reported in healthy children of same age-group from Greece by Sdogou et al (median 46.94 mg/mL in boys and 46.79 ng/mL in girls.) [12]. Dewan et al [13] in their study demonstrated that the mean serum hepcidin levels were 3.03 (1.06) ng/mL in children under 5 years with IDA. Similarly low hepcidin values have been reported in pediatric IDA patients from other parts of the world. Choi et al [10] reported mean hepcidin in 17 Korean children aged 5 months to 17 years as 2.01 (2.3) ng/mL and Jaeggi et al [14] reported mean hepcidin in 54 African infants without inflammation as 1.2 (4.9) ng/mL. The baseline levels observed in the present study in children between 5-12 years were almost three times of that observed in younger children. A similar observation was made by Sdogou et al in their study and they hypothesized that it is likely due to higher demand for iron in younger children due to their higher growth rate [12]. The present study saw a significant rise in hepcidin in response to treatment with levels rising by 7% as early as one day after initiation of oral iron and by 22% after 14 days. Whereas a previous study carried out by Kitsati et al in patients undergoinh hemodialysis, following administration of intravenous iron sucrose observed that hepcidin rises by 25-200% after 15 minutes of administration [5]. Hence, it can be hypothesized that hepcidin responses are more rapid and much higher when iron is administered by parenteral route than by oral route. In a study by Dewan et al serum hepcidin level in under 5 children (30 children with IDA and 30 matched healthy controls) after 3 months of oral iron therapy, increased to 3.61 (1.48) ng/ml, although the rise was statistically insignificant [13]. In contrast, the present study observed that hepcidin levels rise as early as one day after starting oral iron therapy. The most plausible explanation from these two observations might be that hepcidin rises initially in response to oral iron supplementation and the levels normalise by 3 months. Increase in hepcidin observed in this study is much earlier than reticulocytosis, that can be observed only after 5-7 days after starting therapy. The present study also investigated if pre-therapy hepcidin levels can help in predicting the response to oral iron but the percentage of non-responders was very low to carry out this comparison. Also, no significant correlation was observed between pre-therapy hepcidin levels and change in hemoglobin after treatment. This is in contrast to the previous studies carried out in adult children [15,16]. Bregman et al [15] saw that responsiveness to oral iron in IDA can be predicted from patients' baseline hepcidin level as there was a significant difference in mean hepcidin levels between responders (11.3 ng/ml) and non-responders (38.4 ng/mL) ($P < 0.001$). They concluded that hepcidin levels > 20 ng/mL can predict non-responsiveness to oral iron with sensitivity of 41.3%, specificity of 84.4%, and positive predictive value of 81.6%. Wittkamp et al [16] in their study saw that median serum hepcidin levels were significantly lower in patients who responded to intravenous iron [2.07 (0.25, 7.97) ng/ml] in comparison to non-responders [10.62 (3.93, 34.77) ng/mL] ($P = 0.04$).

Hence, we conclude that serum hepcidin shows an early rise in response to oral iron therapy in children with IDA. Research is recommended to ascertain the role of hepcidin as a biomarker to assess early

response to therapy in IDA by comparing its levels in relation to conventional hematological parameters in non-responders and responders.

Ethics clearance: Ref No.TP (MD/MS) (48/2019)/ IEC/ ABVIMS/ RMLH – 712/19; dated Oct 22, 2019.
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WHAT THIS STUDY ADDS?

- Serum hepcidin levels show a significant rise within 24 hours in children with iron deficiency anemia in response to oral iron therapy.

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Table I Laboratory Parameters at Baseline, Day 1 and Day 14 of Oral Iron Therapy (*n* = 64)

Parameters	*Baseline (D0)*	*Day 1*	*Day14*	*P value*[a]	*P value*[b]
Hemoglobin (g/dL)	8.81 (1.22)	9.04 (1.24)	10.9 (1.19)	<0.001	< 0.001
MCV (fL)	73.71 (6.28)	74.92 (6.11)	80.8 (8.2)	<0.001	< 0.001
MCH (pg/cell)	22.1 (3.85)	23.21 (3.13)	26.6 (3)	<0.001	< 0.001
MCHC (g/dL)	28.8 (2.63)	29.75 (2.76)	33 (2.87)	<0.001	< 0.001
RBC count (million/mm^3)	3.83 (0.37)	3.98 (0.42)	4.53 (0.4)	0.001	< 0.001
Serum ferritin (ng/mL)	18 (10.6)	19.62 (11.93)	26 (15.8)	<0.001	< 0.001
Serum iron (µg/dL)	26.03 (6.72)	27.75 (6.35)	37 (9.47)	<0.001	< 0.001
TIBC (µg/dL)	41.2 (47.8)	411.32 (47.35)	333 (63.9)	0.964	< 0.001
UIBC (µg/dL)	357.3 (58.5)	359.92 (55.14)	271 (47.2)	0.107	< 0.001
Transferrin saturation (%)	6.52 (2.16)	6.96 (2.09)	11.6 (4.7)	<0.001	< 0.001
C-reactive Protein (CRP) (mg/dL)	0.6 (0.22)	-	0.5 (0.28)	-	0.023
Serum hepcidin (ng/mL)					
2-15 y (*n* = 64)	7.81 (4.88)	8.38 (4.96)	9.5 (5.2)	< 0.001	< 0.001
2-5 y (*n* = 15)	3.24 (0.91)	3.72 (1.14)	4.83 (1.29)	< 0.001	< 0.001
5-15 y (*n* = 50)	9.09 (4.77)	9.68 (4.83)	10.82 (5.13)	< 0.001	< 0.001
Boys (*n* = 30)	7.34 (4.71)	7.84 (4.64)	8.84 (4.97)	0.680	0.235
Girls (*n* = 34)	8.22 (5.07)	8.85 (5.25)	10.09 (5.4)	0.616	0.145

MCH Mean corpuscular hemoglobin, MCHC Mean corpuscular hemoglobin concentration, MCV Mean corpuscular volume, RBC Red blood cell, TIBC Total iron binding capacity, UIBC Unsaturated iron binding capacity

[a]*Comparison between D0 and D1;* [b]*Comparison between D0 and D14*

Table II Correlation of Baseline Serum Hepcidin (ng/mL) With Change in Various Parameters on Days 1 and 14

Parameter	*On day 1*		*On day 14*	
	Correlation coefficient	*P value*	*Correlation coefficient*	*P value*
Change in hemoglobin (g/dL)	-0.113	0.373	-0.099	0.434
Change in RBC count (million/mm^3)	0.048	0.706	-0.025	0.846
Change in MVC (fL)	0.030	0.815	0.063	0.620
Change in MCH (pg)	-0.058	0.646	0.027	0.832
Change in MCHC (g/dL)	-0.020	0.875	-0.175	0.167
Change in serum ferritin (ng/ml)	-0.029	0.821	-0.058	0.649

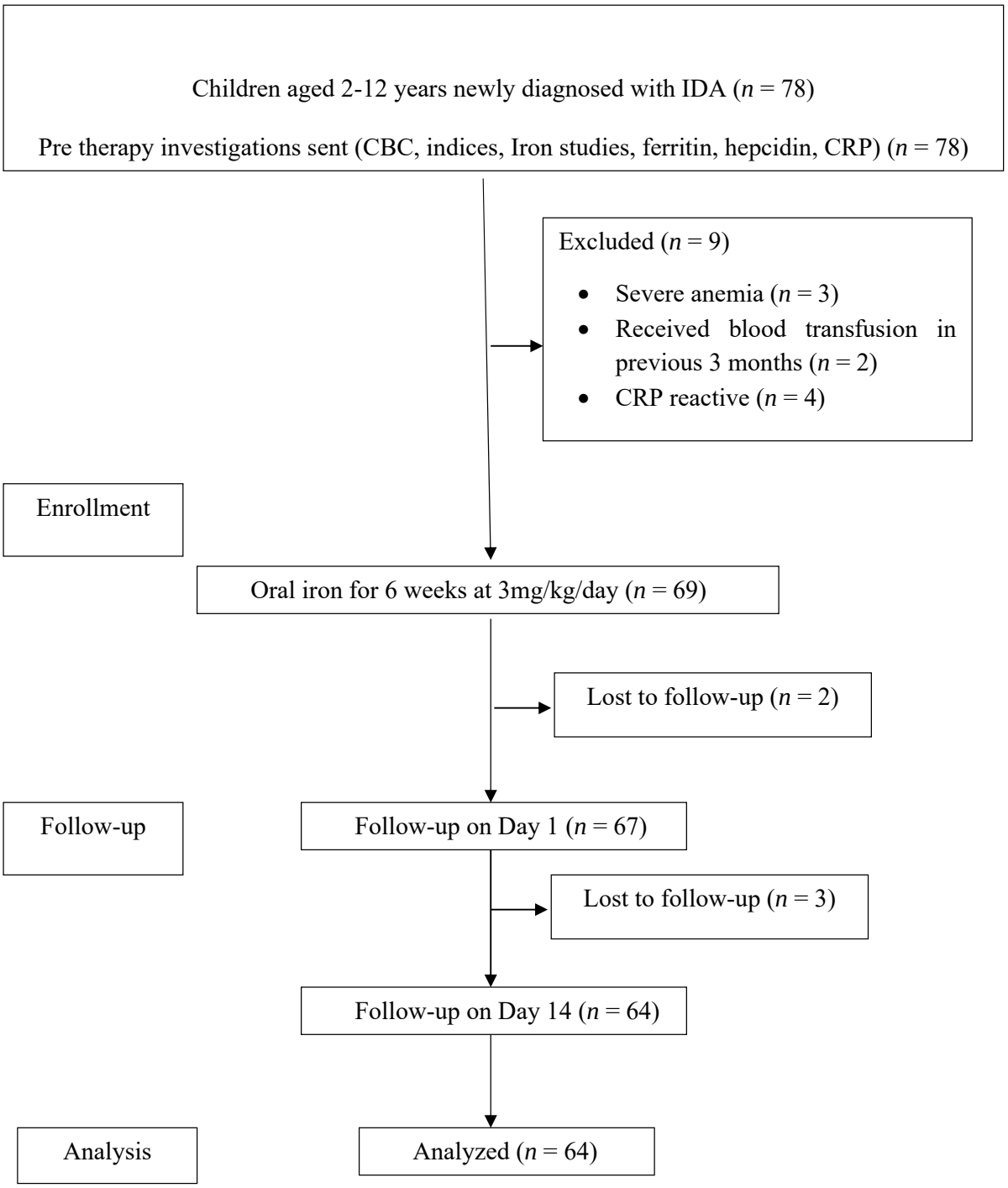

Fig. 1 Flow of participants in the study

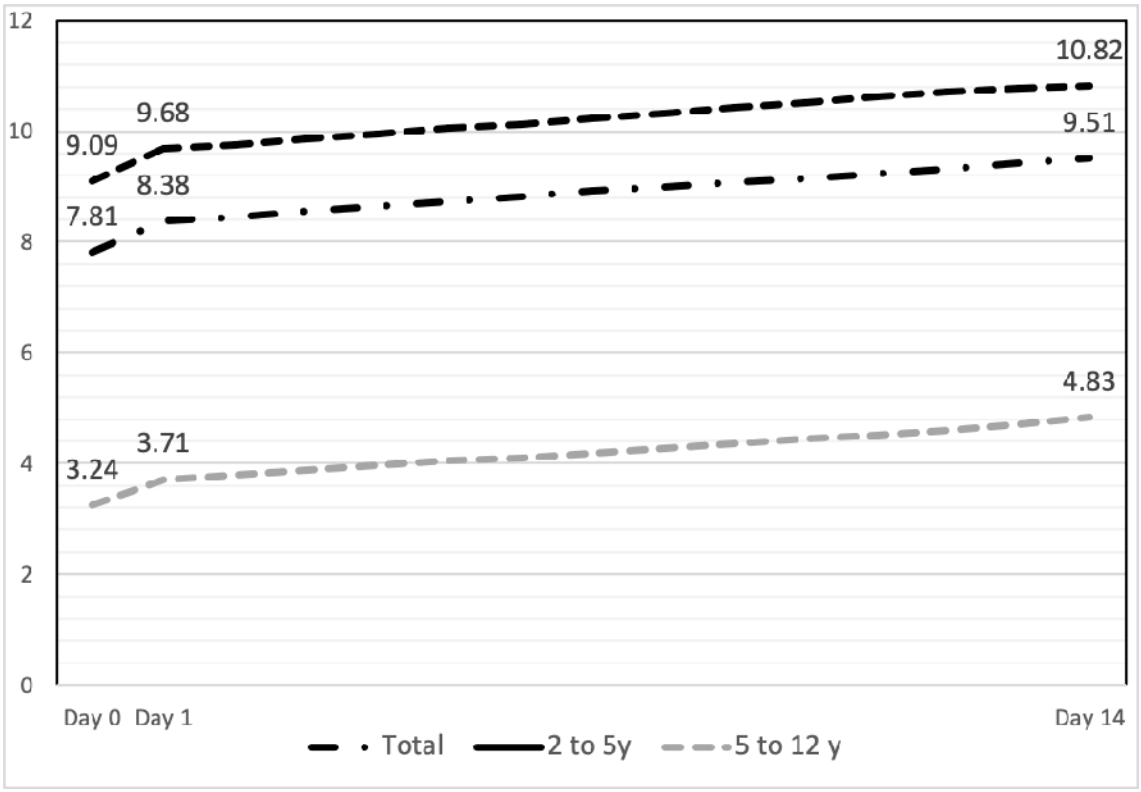

Fig. 2. Serial serum hepcidin levels (ng/mL) following oral iron therapy