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ORIGINAL ARTICLE

Randomized controlled trial of twice-daily versus alternate-day oral iron therapy in the treatment of iron-deficiency anemia

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Abstract

Recent studies in iron-depleted women have challenged the current approach of treating iron-deficiency anemia (IDA) with oral iron in divided daily doses. Alternate day dosing leads to more fractional absorption of iron. In this randomized controlled trial, we looked at the efficacy and safety of alternate-day (AD) versus twice-daily (BD) oral iron in all severity of IDA. Total of 62 patients were randomized, 31 patients in BD arm received 60 mg elemental iron twice daily while 31 patients in AD arm received 120 mg iron on alternate days. The primary endpoint of 2 g/dl rise in hemoglobin was met in significantly more patients in the BD arm at 3 weeks (32.3% vs. 6.5%, $p < 0.0001$) and 6 weeks (58% vs. 35.5%, $p = 0.001$). There was a significant rise in the median hemoglobin at 3 (1.6 vs. 1.1, $p = 0.02$) and 6 weeks (2.9 vs. 2.0 g/dl, $p = 0.03$) in the BD arm. However, the median hemoglobin rise in the AD arm at 6 weeks was not significantly different than the BD arm at 3 weeks. Alternate-day dosing for 6 weeks and twice-daily dosing for 3 weeks resulted in the provision of the same total amount of iron. There were more reports of nausea in the BD arm ($p = 0.03$). In conclusion, the choice of twice-daily or alternate-day oral iron therapy should depend on the severity of anemia, the rapidity of response desired, and patient preference to either regimen due to adverse events. Trial Registration: CTRI reg. no. CTRI/2018/07/015106 http://ctri.nic.in/Clinicaltrials/login.php.

Keywords Iron-deficiency anemia · Oral iron · Daily · Alternate day · Hepcidin

Introduction

Oral iron therapy is the mainstay of treatment of IDA. Current guidelines recommend daily oral iron in divided doses to treat IDA. The standard dosing for oral iron is a single tablet containing 60 mg of elemental iron to be taken two–three times a day [1]. The recent study by Moretti et al. elegantly described what happens with different oral iron dosing and schedules [2]. The morning dose augments the diurnal hepcidin increase in the afternoon and thereby decreases the fractional iron absorption from the afternoon dose. In addition, the afternoon dose leads to another hepcidin increase for 24 h, decreasing the fractional iron absorption from the following morning dose. Alternate-day or weekly dosing leads to more fractional absorption of iron. What is important is that these iron kinetic studies were done in iron-depleted young women and are not necessarily true in all anemic patients. This study challenged the way we should be treating IDA and was the basis of our hypothesis single dosing on alternate days is better than the standard of care twice-daily dosing of oral iron in the treatment of IDA [3]. There are already pre-existing studies on the prevention and treatment of mild IDA in school-going children and pregnant women with intermittent dosing schedules of iron. These studies have shown that

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intermittent dosing schedules are effective in preventing and improving mild IDA [4, 5]. This was also answered in a mechanistic randomized controlled trial in iron-depleted women and in women with mild IDA, which showed higher cumulative fractional and total iron absorption in the alternate-day group than the consecutive-day group at the end of treatment [6]. A follow-up study by the same group showed that iron absorption was greater with alternate-day than with consecutive-day dosing in women with mild IDA [7].

In this randomized, controlled, open-label trial, we looked at the efficacy and safety of once alternate-day schedule compared to the twice-daily schedule in the treatment of all severity of anemia.

Methods

The institutional review board approved the study. Written informed consent was obtained from all the participants before the enrollment. The study was performed in accordance with the Consolidated Standards of Reporting Trials and the declaration of Helsinki. The study was conducted at a single center (tertiary referral center in North India) from July 2017 to December 2018. The study protocol is mentioned in the supplement. The study was registered at the clinical trials registry of India CTRI Reg. no CTRI/2018/07/015106.

Patients of either gender, age > 15 years, and proven iron-deficiency anemia were included in the study. Iron-deficiency anemia was confirmed by serum ferritin level of < 20 ng/ml. Patients with borderline anemia (hemoglobin ≥ 11.5 g/dl), very severe anemia (hemoglobin < 6 g/dl), cardiac failure, pregnancy, and clinical or laboratory evidence of other causes of anemia (iron malabsorption, gastrointestinal bleed, megaloblastic anemia, or anemia of chronic disease) were excluded from the study. Patients were classified as mild anemia (11–11.4 g/dl), moderate anemia (8–10.9 g/dl), and severe anemia (6–8 g/dl) as per the World Health Organization definition [8]. The primary objective of the study was to assess the efficacy of alternate-day oral iron therapy in comparison to twice-daily oral iron therapy in subjects with iron-deficiency anemia. The secondary objective was to assess the adverse events, change in plasma hepcidin (PHep), and RBC parameters in both the arms. The primary endpoint was the proportion of patients achieving ≥ 2 g/dl hemoglobin rise by 3 weeks (day 22) [3, 9] and the quantity of hemoglobin rise by 3 and 6 weeks in both arms. The secondary endpoints were (1) incidence of adverse events, (2) change in plasma hepcidin after 1 week of therapy compared to the baseline, (3) change in reticulocyte hemoglobin content (RHC) after 1 week of therapy compared to the baseline, and (4) change in automated red blood cell indices—mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) after 6 weeks of therapy compared to baseline.

Randomization and masking

One study author assessed the patients for study eligibility (DPL). Another author (RK) was involved in patient randomization, study drug administration, and ensuring compliance. The patients were randomized with a 1:1 allocation in blocks of three to receive either tablet ferrous sulfate 200 mg per oral empty stomach twice-daily (120 mg elemental iron per day) (arm BD: standard arm) or tablet ferrous sulfate 400 mg per oral empty stomach on alternate days (120 mg elemental iron on alternate days) (arm AD: experimental arm). Randomization was stratified by the severity of anemia (mild, moderate, severe as above). The study drug was generic anhydrous ferrous sulfate tablets (Jackson Laboratories, India) of 200 mg, each containing 60 mg elemental iron. Compliance was ensured by weekly phone calls. All the other authors were involved in assessing adverse events and follow-up investigations. All adverse events (AE) subjectively reported by the patients were recorded as per the CTCAE version 4.0 on follow-up visits and weekly phone calls.

Laboratory parameters

The CBC, along with reticulocyte, RHC, and RBC indices, was performed on baseline samples and after 1 week (at day 8). The samples were run on XN-1000® (Sysmex Corporation, Kobe, Japan) automated hematology analyzer. Repeat assessment of hemoglobin was done at 3 and 6 weeks of therapy. Plasma hepcidin was measured at the end of week 1 (day 8) using the Human Hepcidin ELISA Kit (Sincere Biotech, China) based on sandwich ELISA principle as per the manufacturer's instructions. A standard curve was obtained with an intra-run coefficient of variation of 10%. The ELISA run was read on Tecan ELISA reader, providing both optical density and absolute values for hepcidin. The upper and lower detection limits for the kit were 3.12 to 200 ng/ml, with a sensitivity of 0.6 ng/ml.

Statistical analysis

The sample size was estimated based on previously published studies with a difference in the experimental and control arm means of 0.7 g/dl after 30 doses and a standard deviation of 1 g/dl [6] (~ 1 g/dl of hemoglobin after 42 doses), 1:1 allocation, type I error probability of 0.05, and a power of 0.95. This was estimated to be a total of 27 patients in each arm. Assuming a loss to follow-up rate of 15%, the accrual goal was 31 patients per arm. Categorical variables, including the proportion of patients with response and adverse events, were compared using the chi-square test. The Mann-Whitney test was used for the comparison of two groups. Significance was set at $p < 0.05$ for all tests. Prism v7·0 software was used for statistical analysis.

Results

A total of 80 patients were screened for eligibility out of which a total of 62 patients were randomized (31 each in the BD and AD arm; Fig. 1). Three patients in the AD arm did not receive the allocated intervention and were included in the intent to treat analysis. The baseline characteristics of the patients are as mentioned in Table 1. The mean age of the patients was 37.1 and 34.3 years, respectively, in the BD and AD arms. There were predominantly females (83.9% and 91.3% respectively) in both arms. The baseline characteristics were comparable between the two arms. The two arms were matched for age, gender, the severity of anemia, and PHep.

Primary endpoint

The primary endpoint of ≥ 2 g/dl hemoglobin increase was met in significantly more proportion of patients in the BD (32.3%) than in the AD arm (6.5%) at the end of 3 weeks ($p < 0.0001$). The same proportion at the end of 6 weeks was 58% and 35.5%, respectively ($p = 0.001$) (Fig. 2). The median hemoglobin rise at the end of 3 weeks ΔHb (3–0) (1.6 ± 1.2 and 1.1 ± 0.9, $p = 0.02$) and 6 weeks ΔHb (6–0) (2.9 ± 1.7 and 2.0 ± 1.3, $p = 0.03$) was significantly more in the BD arm compared to the AD arm (Fig. 3).

Secondary endpoints

Safety

There were no grade 3/4 AE reported in either arm. None of the patients discontinued the drug due to AE. There were predominant gastrointestinal AE with nausea being the most common. Nausea was reported in significantly more patients in the BD arm (38.7%) compared to patients in the AD arm (22.5%) ($p = 0.03$). The other AE of dyspepsia, vomiting, constipation, and diarrhea were seen in less than 10% of patients in both the arms and were not significantly different (Table 2).

Early response markers

The mean RHC which was comparable at baseline in both the arms was significantly more in the BD arm (28.2 ± 5.3 pg) compared to the AD arm (23.4 ± 4.5 pg) at the end of week 1 ($p = 0.003$) (Fig. 4). The mean rise in RHC (ΔRHC) was significantly more too in the BD compared to the AD arm (5.9 ± 5.7, 2.0 ± 2.2 respectively, $p = 0.004$). The reticulocyte counts peaked at the end of the first week. There was more reticulocytosis in BD arm (1.2 ± 1.0 %) as compared to AD arm (0.38 ± 1.3%) ($p = 0.09$). This did not reflect on the early rise of hemoglobin in the first week. The plasma hepcidin levels did not change significantly in both the arms with the mean change ΔPHep being 0.75 ± 5.7 and 1.2 ±

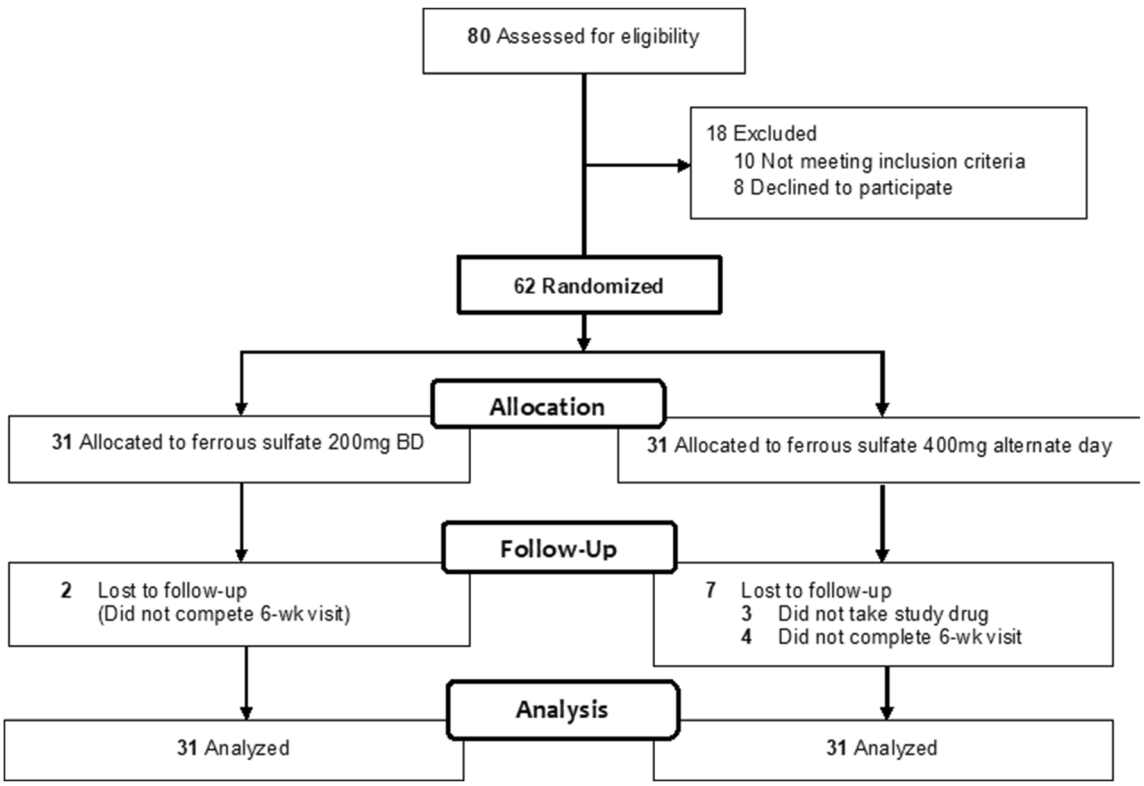

Fig. 1 Enrollment, randomization, and follow-up of the study patients

Table 1 Baseline characteristics in the BD and AD arms

Characteristics		BD (n = 31), mean ± SD	AD (n = 31), mean ± SD
Age (years)		37.1 ± 12.3	34.3 ± 12.4
Gender, n (%)	Female	26 (83.9%)	28 (91.3%)
	Male	5 (16.1%)	3 (9.7%)
Hb (g/dl) all severity anemia		8.9 ± 1.3	8.9 ± 1.6
Hb (g/dl) mild anemia		11.1 ± 0.1	11.2 ± 0.1
Hb (g/dl) moderate anemia		9.1 ± 0.8	9.4 ± 0.9
Hb (g/dl) severe anemia		7.3 ± 0.6	6.9 ± 0.7
MCV (fl)		71.7 ± 6.8	74.3 ± 9.7
MCH (pg)		20.6 ± 2.9	21.8 ± 3.5
Reticulocyte count (%)		1.6 ± 0.7	2.6 ± 4.2
RHC (pg)		21.6 ± 3.8	21.5 ± 5.0
PHep (ng/ml)		28.5 ± 7.1	30.5 ± 7.3
Ferritin (ng/ml)		11.8 ± 8.6	13.1 ± 8.5

BD twice a day, *AD* alternate day, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *RHC* reticulocyte hemoglobin content, *PHep* plasma hepcidin

5.2, respectively, $p = 0.8$ (Fig. 5). There was an increase in PHep in 13 patients in BD, 11 in AD arm, and a decrease in nine patients in BD and eight in AD arm ($p > 0.1$). The RBC indices MCV and MCH were significantly more in the BD arm compared to the AD arm at both the 3- and 6-week time points (data not shown).

Subset analysis

Stratifying by the severity of anemia, the ΔPHep was more in mild anemia patients (3.4 ± 6.0 ng/ml) as compared to moderate-severe anemia patients (0.4 ± 5.3 ng/ml), though this was not significantly different ($p = 0.2$). On subset analysis of moderate and severe anemia together, there was significantly more median hemoglobin rise at the end of three (1.75 vs. 1.2 g/dl respectively, $p = 0.01$) and 6 weeks (3.8 vs. 2.1 g/dl respectively, $p = 0.02$) in the BD compared to the AD arm. The subset analysis of mild anemia showed that the hemoglobin rise was not significantly different in both the arms.

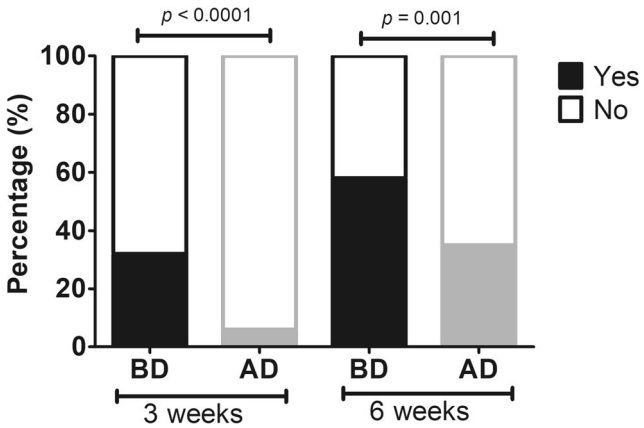

Fig. 2 Percentage of patients attaining primary endpoint of ≥ 2 g/dl rise in hemoglobin

Discussion

In this randomized, controlled, open-label trial, we looked at the efficacy and safety of oral ferrous sulfate once an alternate day (400 mg AD) compared to the standard twice-daily schedule (200 mg BD) in the treatment of all severity of anemia. This is the first such trial in all severity of anemia to the best of our knowledge. We chose the simplest form of oral iron tablets (ferrous sulfate 200 mg—each containing 60 mg elemental iron) since it is available in our national health programs and is cheap and generic. Plus, there is no data to show the superiority of any other formulation of iron tablets over the age-old ferrous sulfate formulation [10]. The schedules were so chosen for the practical ease, compliance, and maintaining at least one standard of care arm (200 mg BD). The basis for this trial was the study by Moretti et al. [2] which challenged the way we should be treating IDA and questioned whether alternate-day dosing would be better than daily dosing [3]. This study was done in iron-depleted young women. Subsequent follow-up studies by the same group have shown that iron absorption was greater with alternate-day than with consecutive-day dosing in women with mild IDA [7]. Whereas the median hemoglobin in this study was 11.3 and 11.6 g/dl and the primary objective was to compare iron absorption, the median hemoglobin in our study was 8.9 g/dl in both arms with a clinically relevant primary endpoint to compare change in hemoglobin.

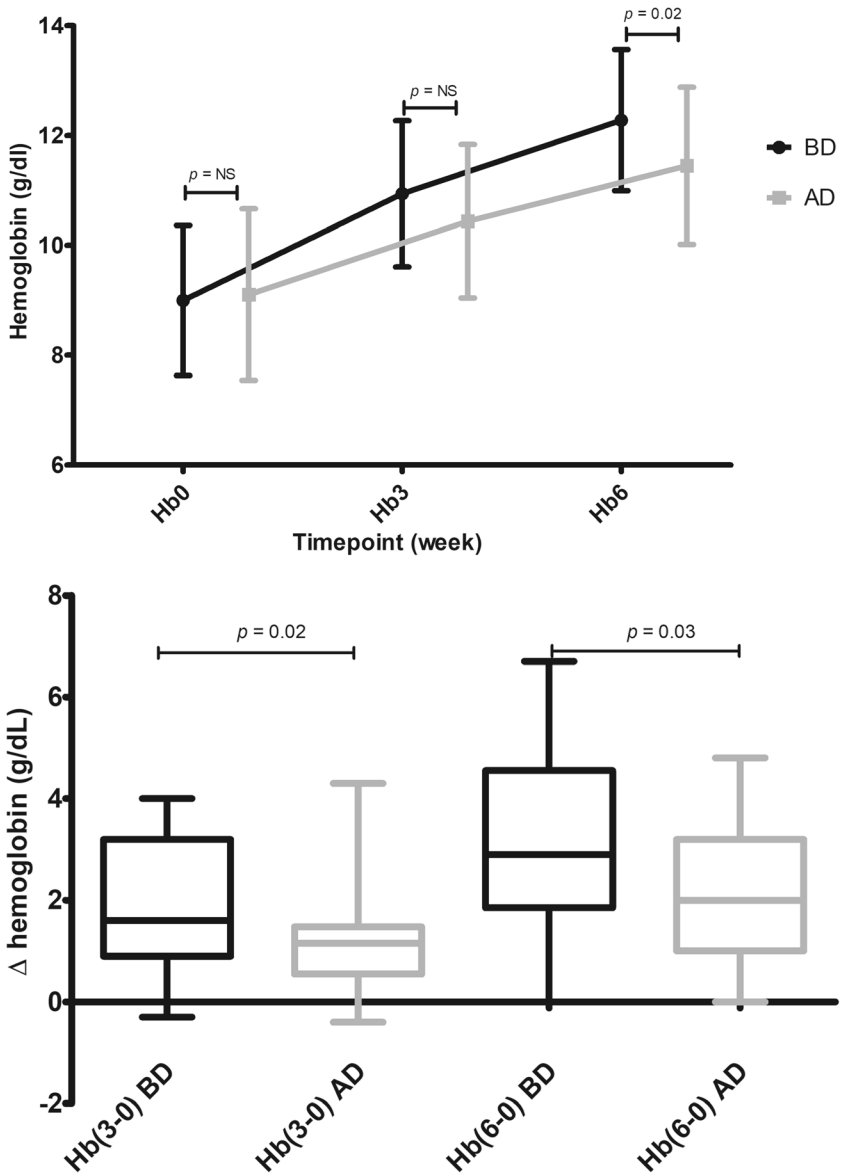

Fig. 3 Hemoglobin in both BD and AD arms in all severity of anemia. **a** Hemoglobin trend over 0 to 6 weeks. **b** Hemoglobin change

Faster response

The primary endpoint of 2 g/dl rise in hemoglobin was achieved in more proportion of patients in the BD arm compared to the AD arm at 3 weeks. The AD arm could not catch up with the BD arm at 6 weeks, as there were still more patients in the BD arm attaining the primary endpoint. However, the proportion of patients at 6 weeks in the AD arm was not significantly different than the patients at 3 weeks in the BD arm. Similarly, the mean rise in hemoglobin though significantly more in the BD arm compared to the AD arm at the end of 3 and 6 weeks respectively, the median rise was not significantly different in the AD arm at 6 weeks compared to the BD arm at 3 weeks. Alternate-day dosing for 6 weeks and twice-daily dosing for 3 weeks resulted in the provision of the same total amount of iron. This implies that BD dosing can attain a faster increase in hemoglobin levels compared to AD dosing at 3 weeks. The speed of response with ≥ 2 g/dl hemoglobin rise by 3 weeks may be important in patients with severe anemia [3, 8].

Better tolerance

The AD arm had a favorable adverse event profile with only nausea being higher in the BD arm compared to the AD arm. The AD dosing will eventually lead to an increase of hemoglobin over a longer period. This happens despite only half of

Table 2 Incidence of adverse events in BD and AD arms over the study period

Adverse event (CTCAE Grade 1-2)	BD (n = 31) (%)	AD (n = 31) (%)	p value
Dyspepsia	2 (6.5%)	3 (9.7%)	NS
Nausea	12 (38.7%)	7 (22.5%)	0.01
Vomiting	1 (3.2%)	1 (3.2%)	NS
Constipation	2 (6.5%)	1 (3.2%)	NS
Diarrhea	2 (6.5%)	3 (9.7%)	NS

BD twice a day, *AD* alternate day, *NS* not significant

the elemental iron delivered in the AD compared to the BD arm. This may be important for patients with mild anemia, where the speed of response is less relevant. For patients with moderate anemia, the dosing strategy can be individualized as per patient preference for faster response or better tolerance.

Early response and hepcidin kinetics

Reticulocyte hemoglobin content (RHC) is the measure of available functional iron over the previous few days for the new RBCs and one of the earliest means of detecting response to treatment. Patients in the BD arm had greater rise in RHC and reticulocytosis than patients in the AD arm, indicating the availability of more iron for erythropoiesis in the BD compared to the AD dosing. The PHep kinetics were not significantly different from the baseline and between the two arms at the end of 1 week. Though the change in PHep was more in mild IDA than moderate to severe anemia, this was insignificant probably due to small patient numbers in the mild IDA subgroup. This is in contrast to the previous study by Stoffel et al., in which serum PHep were uniformly higher in the daily arm than alternate day arm throughout the first 14 days [6]. This finding was demonstrated only in patients who were iron depleted or had mild anemia. Patients in this study were given 60 mg elemental iron on alternate days, which is half of the dose given in the current study. Changes in PHep levels in our study can be attributed to increased erythropoiesis in moderate and severe anemia after oral iron intake. The production of PHep is inhibited by the expansion of erythropoiesis, iron deficiency, and tissue hypoxia [11]. In patients with moderate to severe IDA, the erythropoiesis is geared for expansion in the face of available iron and hence suppresses the increase in hepcidin after the availability of oral iron.

Response stratified by severity of anemia

On subset analysis, the median rise in hemoglobin levels was similar across both the arms in mild anemia. This is as expected from previously published studies [2, 4–6]. This could be due to less strain for erythropoiesis in mild anemia after the availability of oral iron and intact hepcidin axis. This could also be because the rise in hemoglobin in mild anemia is only of a smaller degree to normalization and hence not statistically significant. On the contrary, in moderate-severe anemia, the patients in the BD arm had significantly more median hemoglobin rise than patients in the AD arm at both the time points of 3 (1.7 vs. 1.2, respectively, p = 0.01) and 6 weeks (3.8 vs. 2.1, respectively, p = 0.02, respectively). However, this subset analysis was not part of the planned primary endpoint analysis and the patient numbers are small to draw any conclusions.

In summary, ours is the first study to the best of our knowledge testing the impact of twice-daily versus alternate-day oral

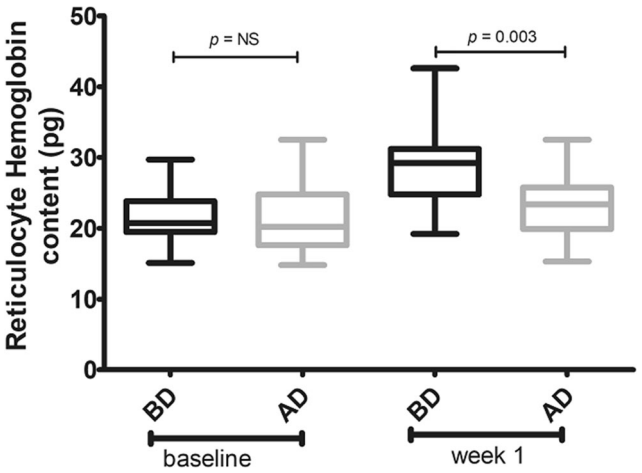

Fig. 4 Change in reticulocyte hemoglobin content (RHC) after 1 week of therapy

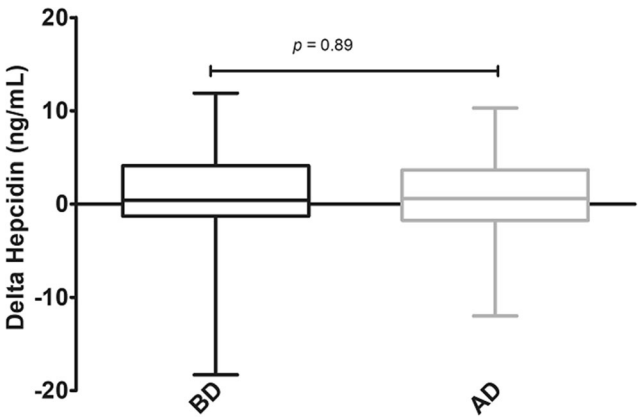

Fig. 5 Change in plasma hepcidin (PHep) after 1 week of therapy

iron therapy in the treatment of all severity of IDA. If the speed of hemoglobin response is desirable, which is the case in severe anemia, twice-daily dosing of oral iron is still better than alternate-day dosing. If the speed of response is not desirable, but tolerance is as is the case in mild anemia, alternate-day iron therapy is better. This could be the preferred choice specifically in preventive strategies or in iron-depleted patients. For patients with moderate anemia, the dosing strategy can be individualized as per patient preference for faster response or better tolerance. Ultimately, the choice of twice-daily or alternate-day oral iron therapy should depend on the severity of anemia, the rapidity of response desired, and patient preference to either regimen due to adverse events.

Limitation

Our study measured PHep levels at a one-time point after therapy. Serial PHep would help understand the kinetics in moderate and severe IDA patients. Future studies should look at comparing twice the daily target dose on alternate days to ensure a comparable cumulative dose of elemental ion is delivered in both arms.

Author contributions DPL, PM, SV, and PB conceived and designed the study. All authors were involved in patient recruitment and clinical care of the patients. PB, RD, and NV analyzed the lab data. DPL, RK, PB, and PM drafted the manuscript. All authors revised the manuscript and approved the final version.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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