International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.352.360

Research Article
Low-Dose Iron is Safer, Tolerable and Equally Effective to High-Dose Iron in Improving Iron Status in Mild-Anemic Female Participants

[1,2]Gaurav Kumar Jain, [1]Anshul Gupta, [3]Abha Mishra, [4]Nilesh Chandra, [5]Musarrat Husain Warsi, [6]Shadab Md, [7]Shahid Karim, [8]Reshma Parekar and [8]Abhishek Dhama

[1]Department of Pharmaceutics, Delhi Pharmaceutical Science and Research University, New Delhi 110017, India
[2]Center for Advanced Formulation Technology, Delhi Pharmaceutical Sciences and Research University, New Delhi 110017, India
[3]Vardhman Mahavir Medical College and Safdarjung Hospital, Ansari Nagar, New Delhi 110029, India
[4]Indian Council of Medical Research, Ansari Nagar, New Delhi 110029, India
[5]Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Taif University, Taif 21944, Saudi Arabia
[6]Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[7]Department of Clinical Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[8]Lupin Limited, Western Expressway Highway, Santacruz-East, Mumbai 400055, India

Abstract

Background and Objective: Despite being mainstay treatment for iron deficiency anemia, use of oral iron is limited due to increased incidence of unwanted GI effects. New evidences suggested that the unwanted effects are predominantly due to excess free or unbound iron in the body and are linked to dose and form of iron. Thus, a pilot clinical study was conducted in female subjects with mild anemia to evaluate the safety, tolerability and efficacy of low-dose iron chelate as an alternative to high-dose iron salt. **Materials and Methods:** A total of 12 subjects were randomized into 2 groups (n = 6) to receive either high-dose iron or low-dose iron, orally once daily for 4 weeks. At baseline and post-treatment, the gastrointestinal (GI) inflammation, C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR) were assessed. For safety, changes in Estimated Glomerular Filtration Rate (e-GFR), Blood Urea Nitrogen (BUN), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were determined. The degree of discomfort from nausea, vomiting, abdominal pain, metallic taste, GI upset and blackening of stools was scored on a scale of 0 to 3. The Hb response, serum-ferritin and total iron binding capacity were determined for iron status. **Results:** The WBC scintigraphy indicated GI inflammation in two out of six subjects in the HDI group (33.3%), however, no inflammation was observed in the LDI group. The post-treatment changes in ESR, e-GFR, BUN, AST and ALT were not significantly different from the baseline in both groups, however, a significant decrease ($p<0.05$) in CRP was observed in both HDI and LDI groups. Symptom-based scoring showed that LDI treatment (0.86) was better tolerated than HDI treatment (1.86). Both treatments showed a comparable rise in Hb. **Conclusion:** The low-dose iron chelate was found to be safer, tolerable and equally effective to high-dose ferrous ascorbate in improving iron status.

Key words: Anemia, ferrous ascorbate, hemoglobin, iron amino acid chelate, WBC scintigraphy, inflammation

Citation: Jain, G.K., A. Gupta, A. Mishra, N. Chandra and M.H. Warsi *et al*., 2024. Low-dose iron is safer, tolerable and equally effective to high-dose iron in improving iron status in mild-anemic female participants. Int. J. Pharmacol., 20: 352-360.

Corresponding Author: Gaurav Kumar Jain, Department of Pharmaceutics, Delhi Pharmaceutical Science and Research University, New Delhi 110017, India

Funding: The Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Saudi Arabia, has funded this project under grant No. (KEP-MSc: 11-166-1443).

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Iron deficiency is one of the key contributors to the global burden of disease and predominantly affects pregnant women, children and low to middle-income populations[1,2]. One of the main consequences of iron deficiency is anemia, which affects nearly 15-20% of world's population[3]. As per National Family Health Survey (2019-21), nearly 67.1% of children, 57.0% of women (15-49 years) and 25.0% of men (15-49 years) are anemic in India[4,5]. Oral iron supplementation is the first line of treatment in most cases. It is a cheap and effective means of increasing Hemoglobin (Hb) and restoring body iron stores. Several iron supplements with varied doses, salt, solubility absorption and chemical state are commercially available[6]. However, a considerable proportion of patients on oral iron suffer from GI side effects and few report GI mucosal injury, resulting in non-adherence to therapy[7,8]. A meta-analysis showed that constipation (12%), diarrhea (8%) and nausea (11%) are common problems with oral iron therapy[9]. The study also showed that the propensity of GI side effects by oral iron is 3-fold higher compared to intravenous iron[10]. The GI inflammation and symptoms occur due to a combination of the formation of reactive oxygen species (ROS) through iron-induced redox cycling in the gut lumen and modification of the microbiota composition[6,11].

Numerous reports compared the efficacy of oral iron supplements through short-term and long-term clinical studies in children with iron deficiency[12,13], pregnant women[14-16] and anemic patients[17]. The efficacy of low-dose iron vs high-dose iron is also reported, however, the debate on the advantage of one over the other is ongoing[18]. Several reports describe the effectiveness and side effect profile of different doses of oral iron supplementation[18,19]. However, reports evaluating the safety of oral iron are limited and are based on recording patient responses[16,17]. One of the reports assesses the safety of oral iron therapy by endoscopy, which is highly invasive and patient-unfriendly[8,20]. Labeled WBCs are widely employed in the diagnosis of inflammatory bowel disease and acute osteomyelitis and recently their role in mediating GI mucosal injury/inflammation has been evident[21,22]. For the very first time, 99mTc-HMPAO labelled WBC scintigraphy to compare the safety of high-dose and low-dose iron treatment. Further, in this study tolerance and Hb response were compared following 4 week treatment with either high-dose ferrous ascorbate or low-dose iron amino acid chelate (IAAC).

MATERIALS AND METHODS

Study design: This open-label, parallel, randomized, pilot clinical study was conducted at Must and More Diagnostic Center (Rohini, New Delhi) in collaboration with a tertiary clinic (Greater Kailash, New Delhi) from December, 2021 to June, 2022.

Ethical consideration: The ethical clearance was obtained from the Independent Ethics Committee, Good Society Ethical Research, Delhi (GSER/2021/BMR-AP/015).

Subjects: A total of twelve female subjects, aged 18-40 years with Hb, 10 to 12 g dL^{-1} were enrolled after obtaining a written informed consent. Subjects with moderate to severe anemia (Hb <10.0 g dL^{-1}) or serum creatinine greater than 1.0 mg dL^{-1} or BUN greater than 18 mg dL^{-1} or with a history of GI diseases or those donated blood or plasma 2 weeks before the study period were excluded. Subjects allergic to iron preparation, taking iron supplements, suffering from iron absorption-related problems, consuming medications interfering with iron absorption and pregnant and lactating females were also excluded.

Protocol and treatment: The subjects befitting the inclusion criteria were enrolled. The demographic details and clinical history were recorded and baseline WBC scintigraphy and blood tests were performed. Thereafter, six subjects (n = 6) were randomly assigned to (a) High-dose iron (HDI) and (b) Low-dose iron (LDI) treatment groups. The HDI group received a ferrous ascorbate tablet (100 mg elemental iron) and the LDI group received iron amino acid chelate (30 mg elemental iron). Each study product was administered orally, once daily, at least 1 hr before a meal for 4 weeks. Consumption of coffee, tea and milk, 1 hr pre and post-administration of study product was restricted. The first dosing was done at the study site and all subsequent administrations were done by the subject herself based on instructions provided by staff and regular check of the same was maintained. Post 4 weeks treatment, WBC scintigraphy and blood tests were performed. Symptom-based responses were also collected every week to evaluate tolerance to treatment.

Study objectives: The primary objective was to determine the safety and tolerance of low-dose iron supplementation. The secondary objective was to evaluate the efficacy in terms of Hb rise. High-dose iron supplementation was used for comparison.

Safety of low-dose iron

GI inflammation by WBC scintigraphy: Scintigraphy with labeled autologous WBCs was utilized for the detection of GI inflammation. The WBCs were labelled *in vitro* with Technetium 99m-Hexamethylpropyleneamine Oxime (99mTc-HMPAO) by a slight modification of a previously reported procedure[23]. Briefly, 40.0 mL of the subject's blood was collected using a 20 G needle and was gently mixed with 8.0 mL of acid citrate-dextrose (ACD) anticoagulant solution. To this, 6.0 mL of 10% HES solution was added and the mixture was stored for about 1 hr to allow sedimentation and separation of erythrocytes. The WBC-rich plasma thus obtained was centrifuged at 150 g for 5 min. The supernatant was removed and the WBC pellet collected was resuspended in 1.0 mL of normal saline. To 1.0 mL of this WBC suspension, 1.0 mL of freshly prepared 99mTc-HMPAO (~300 MBq) was added and the mixture was incubated for 10 min at room temperature to achieve radiolabeling. Thereafter, 3.0 mL of normal saline was added and the mixture was centrifuged at 150 g for 5 min and the supernatant containing unbound 99mTc-HMPAO was removed. The radiolabeled WBCs were collected and the radioactivity was determined using a scintillation counter. The measured amount of 99mTc-HMPAO labelled WBCs (dose ~200 MBq) was resuspended in saline and reinjected into the subject. Planar whole-body sweep and static imaging were performed under a gamma camera (Hawkeye, GE medical system, USA) equipped with a collimator at 3-h post-injection. All the images were acquired for 10 min duration. The scintigraphic images were reviewed and evaluated by a nuclear medicine specialist.

CRP and ESR levels: Pre and post-treatment CRP and ESR levels were estimated to assess the inflammatory responses. The CRP was measured by the immunoturbidimetric method, whereas ESR was measured by Westergren using VES-MATIC 20 ESR system (Diesee Diagnostica, Italy).

Kidney and liver function test: Plasma e-GFR and BUN levels were determined to assess kidney function, whereas plasma AST and ALT were determined for liver function. These tests were conducted at Must and More Diagnostic Center (New Delhi) using NABL approved standard test procedure.

Tolerance of low-dose iron: Tolerance was assessed by structured, self-reported responses. The degree of discomfort from each symptom: (a) Nausea and vomiting, (b) Abdominal pain, (c) Metallic taste and (d) GI upset and blackening of stools was numerically rated by the study subject in one of four categories: (0) Absent, (1) Mild, (2) Moderate or (3) Severe. The responses were collected weekly (4 per subject) and the score for each group was calculated. The symptom was considered (a) Absent, if the mean value is 0; (b) Mild, if the value is less than 0.5; (c) Moderate, if the value is between 0.5 to <1.5; (d) severe if the value between 1.5 to <2.5 and (e) Very severe if value greater than 2.5. The treatment was compared for tolerance based on the total score.

Hb rise of low-dose iron: Pre and post-treatment Hb was estimated using flow cytometry on a Sysmex XN hematology analyzer (Sysmex America Inc., USA) to determine efficacy. The pre and post-treatment serum ferritin and TIBC were also determined using quantitative enzyme-linked fluorescent immunoassay and chemiluminescence using VITROS 5600 (Ortho-Clinical Diagnosis, Inc., USA), respectively.

Statistical analysis: Data was statistically described in terms of Mean±Standard Deviation or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using the Student's t-test for independent samples. The p-value less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS V26.0 (Statistical Package for the Social Science; SPSS Inc., Chicago, Illinois, USA).

RESULTS

Baseline parameters: No statistically significant ($p>0.05$) difference was observed in the mean age of the subjects between the HDI (29.1±3.20) and LDI (28.7±3.87) group. As shown in Table 1, the baseline e-GFR, BUN, AST, ALT, Hb and TIBC did not differ between the groups, however, CRP, ESR and serum ferritin were significantly different ($p<0.05$). Nevertheless, all the baseline parameters, except Hb, were within the normal range (Table 1). Further, baseline WBC scintigraphy showed the absence of GI inflammation in all the subjects (Table 1 and Fig. 1).

Safety of low-dose iron: At baseline, the scintigraphic images of all the subjects were negligible at 3-h in highly perfused organs such as lungs, liver and kidney. Importantly, in none of the subjects, radioactivity was observed in GI at 3-h. This indicates the absence of GI inflammation in all the subjects at baseline. Post-treatment, the scintigraphic images of 2 out of 6 subjects (HS5 and HS6) in the HDI group (33.3%) showed retention of radioactivity in GI at 3-h. This indicates the occurrence of GI inflammation in these two subjects probably

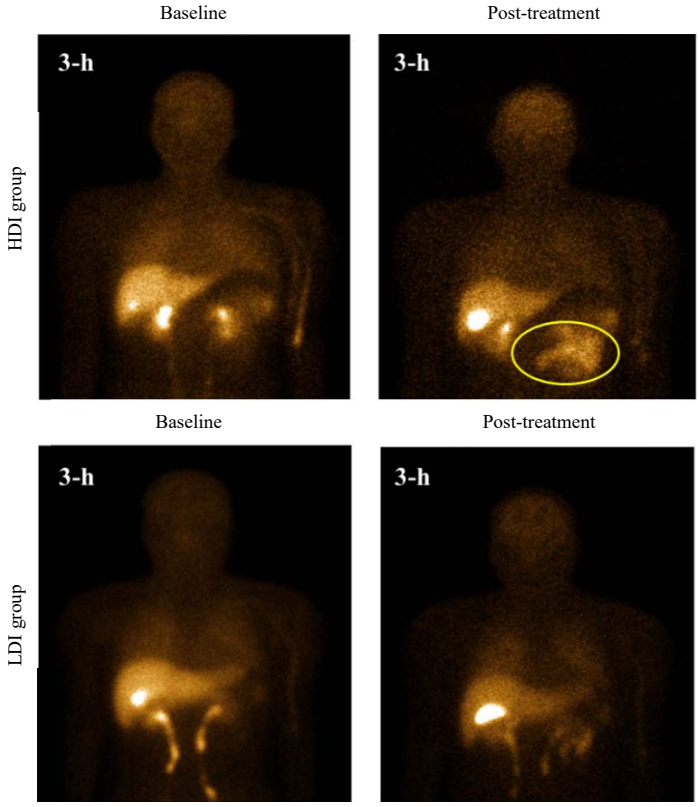

Fig. 1: Representative 3-h baseline and post-treatment anterior scintigraphic image of the individual subject in the HDI and LDI group
Yellow marked area represents GI inflammation

Table 1: Data of the study subjects at baseline and post-treatment with high dose and low dose iron

	HDI group (n = 6)		LDI group (n = 6)	
Parameter	Baseline	Post-treatment	Baseline	Post-treatment
Inflammatory status				
WBC scintigraphy	+0/6	+2/6	+0/6	+0/6
CRP (mg L^{-1})	1.93±0.89	1.45±0.59$^{\#}$	2.38±1.32*	1.91±0.62$^{\#}$
ESR (mm hrs^{-1})	15.17±9.75	13.83±11.21	10.5±5.54*	9.0±4.47
Kidney function				
e-GFR (mL/min/1.73 m^2)	88.73±15.50	87.01±7.64	83.25±16.95	88.5±19.8
Creatinine (mg dL^{-1})	0.95±0.13	0.83±0.14	0.96±0.12	0.92±0.11
BUN (mg dL^{-1})	11.33±1.75	13.16±2.48	11.67±1.2	13.0±1.54
Liver function				
AST (U L^{-1})	33.17±5.56	29.5±6.56	36.50±5.28	28.83±1.72
ALT (U L^{-1})	43.50±13.17	38.16±12.61	40.2±18.3	34.3±8.5
Iron status				
Hb (g dL^{-1})	10.63±0.93	10.95±0.78	10.71±0.82	11.08±0.75
Serum ferritin (µg L^{-1})	132.17±15.28	135.00±65.93	101.7±13.65*	139.6±11.9
TIBC (µg L^{-1})	290.5±42.08	311.0±41.57	311.6±44.24	316.0±30.59

HDI: High-dose iron, LDI: Low-dose iron, WBC: White blood cells, CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate, e-GFR: Estimated Glomerular Filtration Rate, BUN: Blood Urea Nitrogen, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, Hb: Hemoglobin, TIBC: Total Iron-Binding Capacity, +: Number of cases with inflammation, *p<0.05 between groups and $^{\#}$p<0.05 with baseline

owing to high-dose iron consumption for 4 weeks. The low radioactive count of the inflamed GI site in both subjects suggested that the inflammation was mild. Interestingly, retention of radioactivity at 3-h was not evident in any of the subjects in the LDI group (0.0%). Representative baseline and post-treatment scintigraphic images of the HDI group subject (HS5) with retention of radioactivity (inflamed GI) were shown in Fig. 1.

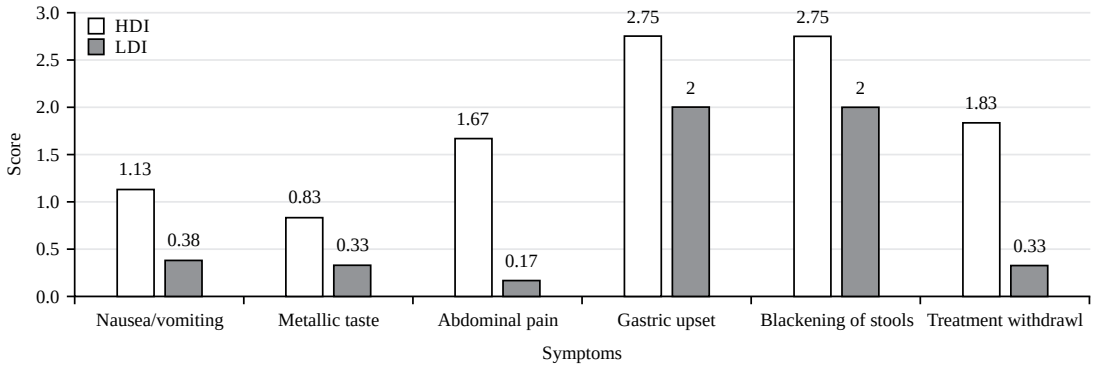

Fig. 2: Symptom-based cumulative response scores of subjects in HDI and LDI groups

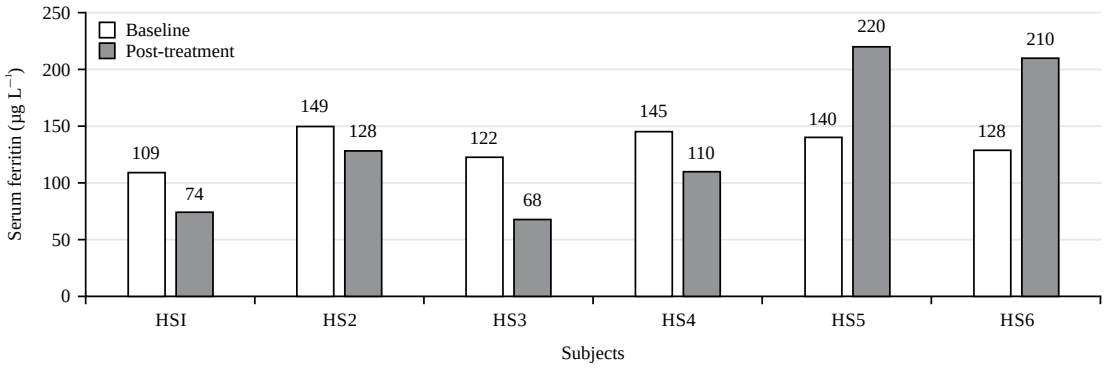

Fig. 3: Baseline and post-treatment serum ferritin concentrations of individual subject in HDI group

Baseline and post-treatment changes in CRP, ESR, e-GFR, creatinine, BUN, AST and ALT are shown in Table 1. Both high-dose and low-dose iron treatment resulted in a small but significant decrease ($p<0.05$) in CRP compared to baseline, however, the difference between the groups was not significant ($p>0.05$). Except for CRP, none of the inflammatory, kidney and liver markers showed any significant ($p>0.05$) change from baseline following treatment with either high-dose or low-dose iron. Further, post-treatment plasma levels of e-GFR, creatinine, BUN, AST and ALT were within the normal limits.

Tolerance of low-dose iron: For assessment of tolerance, symptoms-based questions were numerically rated and cumulative responses were shown in Fig. 2. From the response received it was evident that the symptoms of nausea/vomiting (0.38), metallic taste (0.33) and abdominal pain (0.17) were mild in the group treated with LDI compared to moderate (0.5 to <1.5) in the group treated with HDI sans severe abdominal pain (1.67) in HDI group (Fig. 2). Individual abdominal pain score suggested severe response from HS5 and HS6 subjects, which might be due to inflamed GI as observed during WBC scintigraphy study. In the LDI group, stomach upset and blackening of stools were rated severe (2.0), however, both were rated very severe in HDI group (2.75). As expected, the score for withdrawing the treatment was significantly lower ($p<0.05$) for LDI (0.33) compared to HDI (1.83). Similarly, total mean score for LDI (0.86) was significantly lower ($p<0.05$) than that obtained for HDI (1.86). Of note, no subject discontinued the study because of any adverse symptoms.

Hb rise of low-dose iron: From baseline to 4 weeks, the mean change in Hb level was 0.32 g dL^{-1} in the HDI group and 0.37 in the LDI group (Table 1). Although the Hb rise in the LDI group was 15.6% higher than the HDI group, the difference between the groups was not significant. This study indicates that LDI treatment is equivalent, if not superior to HDI treatment. Further, serum ferritin remained unchanged in both HDI and LDI groups. However, considering individual subjects it was observed that serum ferritin was increased only in two subjects, both with inflamed GI as shown in Fig. 3. Furthermore, no significant rise in TIBC occurred post-treatment with either HDI or LDI and the difference between the groups is insignificant ($p>0.05$).

DISCUSSION

Oral iron supplements tend to develop harmful GI effects including mucosal inflammation and injury, one of the key factors for non-adherence to oral iron supplementation. New evidence suggests that these harmful effects are predominantly due to excess free or unbound iron in the body. Free iron is particularly perilous, as it transits between Fe^{2+} and Fe^{3+} and generates oxidative stress and reactive oxygen species (ROS). Small intestines, being foremost sites for iron absorption are primarily affected by ROS. The ROS causes stress in the endoplasmic reticulum and mitochondria, destruction of cell membranes and onset of intestinal mucosal inflammation and injury[24]. Recently, few authors have tried to establish dose-dependent efficacy and safety and suggested the use of low-dose iron[25], alternate dosing[26] and weekly dosing[27] as a safe and effective means of administrating therapeutic quantity of iron since recommended dietary allowances for iron in healthy adult subjects is 8-18 mg/day and 27 mg/day in pregnant[28].

Nevertheless, this calls for a better understanding of the complexities and thus as a primary objective to compare GI safety and tolerance of high-dose and low-dose iron. Ferrous ascorbate equivalent to 100 mg elemental iron and iron amino acid chelate equivalent to 30 mg elemental iron were used as sources for high-dose and low-dose iron, respectively. Reports related to GI inflammation and iron dose are very limited and those available, have either assessed safety through patient responses[16,17] or the use of invasive and patient unfriendly and uncomfortable endoscopic procedures[8,20]. In the present work, 99mTc-HMPAO labeled autologous WBCs (WBC scintigraphy) are utilized as a sensitive and minimally invasive method for the detection of iron-mediated GI mucosal inflammation.

From the recent literature, it is evident that WBC scintigraphy holds promise for the detection of mucosal infection, lesions and inflammation in patients where other diagnostic tests have failed[29-32]. Since the safety of WBC scintigraphy in anemia and pregnancy has not been established and since the procedure requires the collection of blood and the use of radionuclide, therefore females with moderate to severe anemia and pregnant females were excluded. However, to assess the efficacy (Hb rise) of high-dose and low-dose iron as a secondary objective, we included females with mild anemia (Hb, 10 to 12 g dL^{-1}).

To assess GI inflammation, the scintigraphic images were captured at 3-h post-IV administration of radiolabeled WBC. In normal conditions, it is expected that radiolabeled WBC migrate rapidly to highly perfused organs resulting in intense radioactivity at 15 min in lungs, liver, spleen, kidney and RES followed by distribution throughout the body resulting in scattered diminished. Later at 3-h, the observance of radioactivity becomes negligible owing to radioactive decay. Similar observations were found in baseline and post-treatment scintigraphic images of the current study except for post-treatment in 2 subjects (HS5 and HS6) in the HDI group where radioactivity was retained in the GI tract as could be seen in the 3-h image. The retention is indicative of inflammation of the GI tract and chemotactic attraction of radiolabeled WBCs towards inflamed/injured tissue. Such observations in the HDI group are consistent with previous reports where excess iron is known to cause ROS-mediated intestinal mucosal inflammation/injury[24]. The low radioactive count of the inflamed GI site in both subjects suggested that the inflammation was mild. Further, the absence of inflammation in the LDI subjects strengthens previous reports claiming the safety of low-dose iron[14]. Nonetheless, in agreement with previous reports, present results also demonstrated that low-dose iron chelate was safer than high-dose ferrous ascorbate[14,33].

Oral iron is notorious for its side effects, namely nausea/vomiting, abdominal pain, metallic taste, stomach upset and blackening of stools, limiting its adherence with a rate hovering from 40-60%[9]. These studies showed that LDI was more tolerable than HDI and the adverse symptoms were relatively less in the LDI group. The present observations were comparable with previous reports that showed low tolerability and severe GI effects of high-dose iron[34]. Besides low dose, the existence of IAAC in the neutral form in the gut unlike ionic ferrous ascorbate is advantageous. Of note, subjects with inflamed GI (HS5 and HS6) reported severe abdominal pain and a feeling of withdrawing from the treatment. Nevertheless, no subject discontinued the study because of any of above mentioned or other adverse symptoms.

To further elucidate the inflammatory status, CRP and ESR levels post-treatment were evaluated. Unexpectedly, CRP levels decreased whereas ESR levels remained unchanged post-treatment in both the groups. Although CRP and ESR are inflammatory markers their role in mild GI mucosal inflammation is not clear and present results suggested that both CRP and ESR are poorly correlated with mild GI inflammation occurred due to iron overdose. The decrease in CRP level in HDI-treated subjects remained inexplicable however, maintenance of hemostasis by iron could have played a role. No significant change in eGFR, creatinine, BUN, AST and ALT, post-treatment, suggested that both doses were safe for 4 weeks administration.

Post-treatment Hb rise was observed in both groups. Unexpectedly, the Hb rise in the LDI group was equivalent to the HDI group. It appeared that even with low doses, considerably more quantity of iron was absorbed from IAAC, responsible for equivalent Hb response and low GI side-effects. Several other studies also reported higher iron absorption into intestinal mucosa from IAAC compared with inorganic iron salts[35,36].

A small increase in serum ferritin in the LDI group suggested the superior efficacy of low-dose IAAC compared to high-dose ferrous ascorbate. Mild anemic conditions and only 4 week therapy might be responsible for a small increase in serum ferritin[37]. In the HDI group, the increase was observed only in subjects with inflamed GI and all the remaining subjects showed decreased serum ferritin. Increased serum ferritin in inflammatory conditions has been elucidated previously[38,39]. Unlike serum ferritin, TIBC remained unchanged with either HDI or LDI treatment might be due to the inclusion of subjects with only mild anemia. Though this study highlights the superior safety, tolerability and equivalent Hb rise of low-dose IAAC, but is limited by a small number of research subjects, involving non-pregnant subjects and subjects with mild anemia and treatment restricted for 4 weeks only.

CONCLUSION

The WBC scintigraphy proved to be a minimally invasive method for the determination of drug-induced mild inflammation in GI. Current study concluded that low-dose IAAC (30 mg) was safer, tolerable and equivalent effective compared to high-dose ferrous ascorbate. The low dose might result in a lower fraction of unabsorbed iron which particularly is responsible for adverse effects. Neutral chelated iron and amino acid-mediated gut absorption seem to be responsible for the rise in Hb. Though low-dose iron seems to be an effective supplement, randomized confirmatory studies are needed to confirm and validate the findings of the present study.

SIGNIFICANCE STATEMENT

Despite being the mainstay treatment for iron deficiency anemia, use of oral iron is limited due to the increased incidence of unwanted GI effects. New evidence suggests that the unwanted effects are predominantly due to excess free iron in the body and are linked to the dose and form of iron. Thus, this research was intended to evaluate the safety, tolerability and efficacy of low-dose iron chelate as an alternative to high-dose iron salt. For the first time, WBC scintigraphy was used to determine iron initiated GI inflammation/ injury. Further, tolerance and Hb response was ascertained following 4 weeks supplementation. Our results demonstrated that low-dose IAAC (30 mg) was safer, tolerable and equivalent effective compared to high-dose ferrous ascorbate.

ACKNOWLEDGMENT

The authors are thankful to DSIR-DPSRU-CRTDH Centre for Advanced Formulation Technology for providing research facilities, Acires Labs LLP for Study Management and Lupin Limited for supplying clinical supplies and extending help in clinical studies.

REFERENCES

1. Weyand, A.C., A. Chaitoff, G.L. Freed, M. Sholzberg, S.W. Choi and P.T. McGann, 2023. Prevalence of iron deficiency and iron-deficiency anemia in US Females aged 12-21 years, 2003-2020. JAMA, 329: 2191-2193.
2. Safiri, S., A.A. Kolahi, M. Noori, S.A. Nejadghaderi and N. Karamzad *et al.*, 2021. Burden of anemia and its underlying causes in 204 countries and territories, 1990-2019: Results from the Global Burden of Disease Study 2019. J. Hematol. Oncol., Vol. 14. 10.1186/s13045-021-01202-2.
3. Whittaker, P., 2017. Iron Deficiency and Its Prevention. In: International Encyclopedia of Public Health, Quah, S.R. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780128037089, pp: 342-345.
4. Derman, R.J., S.S. Goudar, S. Thind, S. Bhandari and Z. Aghai *et al.*, 2021. RAPIDIRON: Reducing anaemia in pregnancy in India-A 3-arm, randomized-controlled trial comparing the effectiveness of oral iron with single-dose intravenous iron in the treatment of iron deficiency anaemia in pregnant women and reducing low birth weight deliveries. Trials, Vol. 22. 10.1186/s13063-021-05549-2.
5. Kalaivani, K. and P. Ramachandran, 2018. Time trends in prevalence of anaemia in pregnancy. Indian J. Med. Res., 147: 268-277.
6. Jimenez, K., S. Kulnigg-Dabsch and C. Gasche, 2015. Management of iron deficiency anemia. Gastroenterol. Hepatol., 11: 241-250.
7. Haig, A. and D.K. Driman, 2006. Iron-induced mucosal injury to the upper gastrointestinal tract. Histopathology, 48: 808-812.
8. Kaye, P., K. Abdulla, J. Wood, P. James, S. Foley, K. Ragunath and J. Atherton, 2008. Iron-induced mucosal pathology of the upper gastrointestinal tract: A common finding in patients on oral iron therapy. Histopathology, 53: 311-317.

9. Tolkien, Z., L. Stecher, A.P. Mander, D.I.A. Pereira and J.J. Powell, 2015. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: A systematic review and meta-analysis. PLoS ONE, Vol. 10. 10.1371/journal.pone.0117383.
10. Das, S.N., A. Devi, B.B. Mohanta, A. Choudhury, A. Swain and P.K. Thatoi, 2020. Oral versus intravenous iron therapy in iron deficiency anemia: An observational study. J. Fam. Med. Primary Care, 9: 3619-3622.
11. Yilmaz, B. and H. Li, 2018. Gut microbiota and iron: The crucial actors in health and disease. Pharmaceuticals, Vol. 11. 10.3390/ph11040098.
12. Allen, S., M.K.H. Auth, J.J. Kim and B. Vadamalayan, 2021. Safety, tolerability, and pharmacokinetics of oral ferric maltol in children with iron deficiency: Phase 1 study. JPGN Rep., Vol. 2. 10.1097/PG9.0000000000000090.
13. Maguire, J.L., G. deVeber and P.C. Parkin, 2007. Association between iron-deficiency anemia and stroke in young children. Pediatrics, 120: 1053-1057.
14. Abdel Moety, G.A.F., A.M. Ali, R. Fouad, W. Ramadan, D.S. Belal and H.M. Haggag, 2017. Amino acid chelated iron versus an iron salt in the treatment of iron deficiency anemia with pregnancy: A randomized controlled study. Eur. J. Obstet. Gynecol. Reprod. Biol., 210: 242-246.
15. Jose, A., R. Mahey, J.B. Sharma, N. Bhatla, R. Saxena, M. Kalaivani and A. Kriplani, 2019. Comparison of ferric carboxymaltose and iron sucrose complex for treatment of iron deficiency anemia in pregnancy-randomised controlled trial. BMC Pregnancy Childbirth, Vol. 19. 10.1186/s12884-019-2200-3.
16. Samsudin, S., M. Dulasi, S. Sany, K. Balanathan, S.E. Chong and A. Ali, 2020. Safety and efficacy of intravenous iron sucrose versus low molecular weight iron dextran for treatment of iron deficiency anemia in pregnancy: A randomized controlled trial. Int. J. Women's Health, 12: 1259-1270.
17. Lo, J.O., A.E. Benson, K.L. Martens, M.A. Hedges and H.S. McMurry *et al*., 2023. The role of oral iron in the treatment of adults with iron deficiency. Eur. J. Haematol., 110: 123-130.
18. Yadav, K., M.C. Arjun, O.M. Jacob, S. Kant, F. Ahamed and G. Ramaswamy, 2020. Comparison of different doses of daily iron supplementation for anemia prophylaxis in pregnancy: A systematic review. J. Fam. Med. Primary Care, 9: 1308-1316.
19. Bloor, S.R., R. Schutte and A.R. Hobson, 2021. Oral iron supplementation-gastrointestinal side effects and the impact on the gut microbiota. Microbiol. Res., 12: 491-502.
20. Clere-Jehl, R., E. Sauleau, S. Ciuca, M. Schaeffer and A. Lopes *et al*., 2016. Outcome of endoscopy-negative iron deficiency anemia in patients above 65: A longitudinal multicenter cohort. Medicine, Vol. 95. 10.1097/MD.0000000000005339.
21. Charron, M., F.J. del Rosario and S.A. Kocoshis, 1999. Pediatric inflammatory bowel disease: Assessment with scintigraphy with ^{99m}Tc white blood cells. Radiology, 212: 507-513.
22. Dailey, T.A., M.D. Berven and P.J. Vroman, 2014. ^{99m}Tc-HMPAO-labeled WBC scan for the diagnosis of chronic recurrent multifocal osteomyelitis. J. Nucl. Med. Technol., 42: 299-301.
23. de Vries, E.F.J., M. Roca, F. Jamar, O. Israel and A. Signore, 2010. Guidelines for the labelling of leucocytes with ^{99m}Tc-HMPAO. Eur. J. Nucl. Med. Mol. Imaging, 37: 842-848.
24. Malesza, I.J., J. Bartkowiak-Wieczorek, J. Winkler-Galicki, A. Nowicka and D. Dzięciołowska *et al*., 2022. The dark side of iron: The relationship between iron, inflammation and gut microbiota in selected diseases associated with iron deficiency anaemia-A narrative review. Nutrients, Vol. 14. 10.3390/nu14173478.
25. Makrides, M., C.A. Crowther, R.A. Gibson, R.S. Gibson and C.M. Skeafff, 2003. Efficacy and tolerability of low dose iron supplements during pregnancy: A randomized controlled trial. Am. J. Clin. Nutr., 78: 145-153.
26. Pasupathy, E., R. Kandasamy, K. Thomas and A. Basheer, 2023. Alternate day versus daily oral iron for treatment of iron deficiency anemia: A randomized controlled trial. Sci. Rep., Vol. 13. 10.1038/s41598-023-29034-9.
27. Viteri, F.E., F. Ali and J. Tujague, 1999. Long-term weekly iron supplementation improves and sustains nonpregnant women's iron status as well or better than currently recommended short-term daily supplementation. J. Nutr., 129: 2013-2020.
28. Camaschella, C., 2015. Iron-deficiency anemia. N. Engl. J. Med., 372: 1832-1843.
29. Dolezal, J. and M. Kopacova, 2013. Radionuclide small intestine imaging. Gastroenterol. Res. Pract., Vol. 2013. 10.1155/2013/861619.
30. Davison, S.M., S. Chapman and M.S. Murphy, 2001. ^{99m}Tc-HMPAO leucocyte scintigraphy fails to detect Crohn's disease in the proximal gastrointestinal tract. Arch. Dis. Childhood, 85: 43-46.
31. Grahnquist, L., S.C. Chapman, S. Hvidsten and M.S. Murphy, 2003. Evaluation of ^{99m}Tc-HMPAO leukocyte scintigraphy in the investigation of pediatric inflammatory bowel disease. J. Pediatr., 143: 48-53.
32. Aydın, F., A.K. Cengiz and F. Güngör, 2012. Tc-99m labeled HMPAO white blood cell scintigraphy in pediatric patients. Mol. Imaging Radionuclide Ther., 21: 13-18.
33. Makled, A.K., W.M. Abuelghar, A.A. El-Shahawy and M.A.E. Elshazly, 2020. Amino acid chelated iron versus ferrous fumarate in the treatment of iron deficiency anemia with pregnancy: Randomized controlled trial. Evidence Based Women's Health J., 10: 95-103.
34. Lindblad, A.J., C. Cotton and G.M. Allan, 2015. Iron deficiency anemia in the elderly. Can. Fam. Physician, 61: 159-159.
35. deWayne Ashmead, H., 2001. The absorption and metabolism of iron amino acid chelate. Arch. Latinoamericanos Nutricion, 51: 13-21.

36. Christensen, J.M., M. Ghannam and J.W. Ayres, 1984. Effects of divalent amino acids on iron absorption. J. Pharm. Sci., 73: 1245-1248.
37. Dignass, A., K. Farrag and J. Stein, 2018. Limitations of serum ferritin in diagnosing iron deficiency in inflammatory conditions. Int. J. Chronic Dis., Vol. 2018. 10.1155/2018/9394060.
38. Kell, D.B. and E. Pretorius, 2014. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics, 6: 748-773.
39. Moreira, A.C., G. Mesquita and M.S. Gomes, 2020. Ferritin: An inflammatory player keeping iron at the core of pathogen-host interactions. Microorganisms, Vol. 8. 10.3390/microorganisms8040589.