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Chronic Severe Iron Deficiency Anemia in Hospitalized Indian Patients: Laboratory Profile and Treatment Outcomes

Bhumika Vaishnav[1], Arvind Bamanikar[2], Sukanya Dasgupta[3], Sameer Shaikh[3]

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Author's Affiliation:
[1]Asso. Prof; [2]Professor and Head of Unit; [3]Postgraduate student, Department of Medicine, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India

Correspondence
Dr. Bhumika Vaishnav
bhumika.dholakia@gmail.com

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ABSTRACT

Introduction: Iron deficiency anemia is a common finding which adds to the morbidity and mortality burden in hospitalized patients. This study was done to evaluate the laboratory parameters and different treatment options for chronic severe iron deficiency anemia.

Methods: A cross-sectional study was done on patients with chronic, untreated, severe anemia with hemoglobin (Hb) less than 8g% and a hypochromic microcytic peripheral smear. Blood indices, reticulocyte count, iron studies and bone marrow examination were done.All patients were treated either with oral or parenteral iron and the outcome was evaluated a day before discharge and 28 days post-treatment by repeating the hemogram and a six-minute walk test.

Results: 148 anemic patients were initially enrolled. 73 patients were excluded as their peripheral smear did not show hypochromic, microcytic picture. The remaining 75 were included. Average Hb on admission was 6.9 ± 1.74g/dL. Average serum ferritin levels of 17.0 ± 3.50 ng/ml. Depleted bone marrow iron stores were found in 69.45% cases. Rise in hemoglobin and improvement in functional capacity of patients at 28 days post treatment with parenteral iron was statistically significantly higher than that after oral iron therapy.

Conclusion: Serum ferritin and bone marrow iron stores were sensitive indicators of IDA. Hemoglobin and functional capacityof patients treated with parenteral iron improved rapidly compared to oral iron.

Key words: Anemia, bone marrow, ferritin, iron deficiency

INTRODUCTION

Anemia is functionally defined as an insufficient red blood cell (RBC) mass to adequately deliver oxygen to peripheral tissues.[1] Anemias can be broadly classified as hypoproliferative or hyperproliferative types.[2] Iron deficiency anemia (IDA) can be classified as a hypoproliferative anemia with or without abnormal iron metabolism. Iron deficiency is one of the most prevalent forms of malnutrition worldwide.[3] About 50% of all cases of anemia are due to iron deficiency.[4] Globally, about 850,000 deaths are attributable to it annually, most of them in Africa and parts of Asia.[5] It is a common medical problem and is associated with an increase in morbidity and mortality in special populations such as the elderly, pregnant females, patients with chronic renal disease etc. [6,7,8]

Nutritional deficiency is the most common cause of IDA in India.[9] As per the World Health Organization (WHO), when the hemoglobin concentration is less than 8 g/dL, it is classified as severe anemia.[10] Severe anemia in hospitalized patients worsens

their morbidity and mortality profile and increases the duration of hospital stay.[11,12,13]

This study was done with an objective to analyze the laboratory data of chronic, untreated, severe IDA amongst hospitalized patients and to observe and compare the treatment outcomes with oral and parenteral hematinic therapy.

METHODS

This was a cross-sectional observational and analytical study conducted at a tertiary care hospital in Pune, Maharashtraover a period of six monthsbetween November 2015 and May 2016. 148 adult patients admitted to the hospital with chronic, untreated severe anemia – (Hemoglobin < 8 g/dL) were initially enrolled in the study.Patients with hypochromic microcytic type of peripheral picture with low RBC indices were presumed to be having iron deficiency and were taken as study subjects. A total of 75 patients were identified in this group and were included in the final study after a detailed (about the possible adverse effects) and informed consent from the participants. Ethical committee approval was also taken prior to the commencement of the study.

Exclusion criteria included: age less than 18 years; acute blood loss due to any cause in last 8 weeks; pregnancy; chronic hepatic and renal diseases; and diagnosed cases of hemoglobinopathy and malignancy. All patients with evidence of acute / chronic inflammation (high leucocyte count and positive C-Reactive Protein (CRP)) were also excluded from the study.

Complete clinical examination was carried out on all patients. Hemoglobin (Hb g/dl), erythrocyte count (/cumm), RBC indices (Mean Corpuscular Volume (MCV) (fl), Mean Corpuscular Hemoglobin (MCH) (pg), Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dL), reticulocyte count (%), and Red cell Distribution Width (RDW) (%)) were measured on the blood collected in the Ethylenediaminetetraacetic acid (EDTA) vials for all the patients. Reticulocyte Production Index (RPI) was calculated using the following formula:

$$\text{RPI} = \frac{(\text{Retic count X Hemoglobin (observed)})}{\text{Normal hemoglobin X } 0.5}$$

The following laboratory values were measured by collecting the blood in plain vials: Serum Iron (µg/dL) by Ferrozine method without deproteinization; Serum Ferritin (ng/ml) by fully automated bidirectionally Interfaced Chemi Luminescent Immunoassay; Total Iron Binding Capacity (TIBC in µg/dL) by Spectrophotometric assay; and Transferrin Saturation (TSAT in %) calculated as a ratio of serum iron and TIBC multiplied by 100.

Bone-marrow aspiration under aseptic precautions was carried out on patients who gave consent for the same (72 out of 75). The bone marrow samples were stained with the Wright - Giemsa stain along with Prussian blue stain (for estimation of iron stores).Once the diagnosis of IDA was confirmed, patients were given treatment in the form of either injectable iron formulation(ferric carboxymaltose) or oral iron supplementation. The decision regarding the choice of treatment was taken by the respective treating physicians, who were not co-authors of this study. Both preparations were given as per the standard dosage schedule (i.e oral iron was given daily in 2-3 divided doses so that a total of 200 mg of elemental iron per day entered the body and parenteral iron was given on a single day after calculating the total iron dose using the following formula:- Body weight (kg) X 2.3 X (15 – patient's hemoglobin, g/dL) + 500mg (for stores)).

Hemoglobin on the day of admission, on the day before discharge and at one-month follow up was checked. Clinical improvement in the patients was assessed by a Six-Minute Walk (SMW) test on the day of admission and at one month after that.

Age, body weight, initial laboratory parameters, and results of iron studies for the study subjects were noted as average (mean) ± two standard deviations. Average Hb and average distance covered in SMW test were compared between the subgroups administered parenteral iron and oral iron by applying two-tailed Welch's t-test, as the variances of the two samples were unequal. Similarly, within each of the subgroups administered parenteral iron and oral iron, average Hb and average distance covered in SMW test were compared between the day of admission and the 28th day from the day of admission, respectively, using two-tailed Welch's t-test. All statistical tests were conducted at a 95% confidence level, so that a P-value of less than 0.05 was considered statistically significant. Statistical tests were conducted using Microsoft Excel 2013.

RESULTS

Out of 148 indoor patients with severe anemia, 75 patients had hypochromic microcytic anemia on peripheral smear and were further investigated (n = 75). Among these patients, 26 were males and 49 were females. Average age of the patients was 40 ± 14.8 years. All the results are of the day of admission. Pulse rate of the patients was 78 ± 14 beats per minute and Body Mass Index (BMI) was 22 ± 3.2 kg/m^2.Average hemoglobin was 6.8 ± 1.71 g/dL. The averageReticulocyte Production Index(RPI or corrected reticulocyte count) was 0.3. Table 1 shows gender-wisedemographic profile

and initial laboratory evaluation results of the patients.Average Hematocrit values were 35.8 ± 4.45%. Mean Corpuscular Volume was low (MCV was 71.3 ± 21.5 fL, normal level – 80-100 fL), Mean Corpuscular Hemoglobin was low (MCH was 16.9 ± 5.6 pg, normal level – 27-34 pg), Mean Corpuscular Hemoglobin Concentration was also low (MCHC was 23.6 ± 4.7 gm/dL, normal level – 32-36 gm/dL).

Table 1: Demographic and initial laboratory parameters of the study participants

Parameter	Observed Values (Mean ± 2SD		Normal Reference Range**
	Male (n = 26)*	Female (n = 49)*	
Age (years)	49 ± 18	36 ± 30.2	
Body weight (kg)	53.6 ± 20.0	42.1 ± 15.6	
Hemoglobin on day 1 of admission (g/dL)	6.9 ± 1.74	6.8 ± 1.70	12-16
Red cell distribution width (RDW) (%)	18.9 ± 4.7	19.4 ± 6.7	11 – 16%
Hematocrit (%)	36.0 ± 4.42	35.8 ± 4.48	36 – 54%
Reticulocyte count (%)	1.0 ± 0.7	1.2 ± 0.8	1.5 – 2.5%
Blood Indices			
Mean Corpuscular Volume – MCV (fL)	70.6 ± 15	72.9 ± 28.5	80-100
Mean corpuscular Hb – MCH (pg)	17.3 ± 5.4	16.6 ± 5.8	27.0 – 34.0
Mean corpuscular Hb concentration MCHC (g/dL)	24.0 ± 5.0	23.3 ± 4.36	32.0 – 36.0

*Mean ± 2 Standard Deviations; **Range

Table 2: Results of Iron studies carried out on patients with severe anemia

Parameter	Males (n = 26)*	Females (n = 49)*	Normal range**
Iron (µg/dL)	48.1 ± 30.2	41.7 ± 32.4	60 -180
Total Iron Binding Capacity (TIBC) (µg/dL)	287.2 ± 52.5	284.9 ± 63.9	215 - 535
% Transferrin saturation (%)	16.99 ± 11.6	14.88 ± 12.6	13 -45
Ferritin (ng/ml)	17.1 ± 6.84	17.0 ± 7.08	10 - 322

*Mean ± 2 Standard Deviations; **Range

Table 3: Comparison of Average Hb and Results of 6-Minute Walk Test between Day of admission and Day 28 post treatment to the hospital when treated with Oral Iron and Parenteral Iron

Indicators	Oral Iron (n=20)	Parenteral Iron (n = 55)
Average Hemoglobin (g/dL)		
Day of admission	6.7 ± 1.6	6.9 ± 1.6
Day 28post treatment	8.5 ± 1.8	9.6 ± 1.9
P value	<0.001	<0.001
6-Minute Walk Test (Distance (m))		
Day of admission	203 ± 90	241 ± 98
Day 28post treatment	216 ± 88	241 ± 98
P value	0.38	< 0.001

Statistical test applied – Welch's t-test

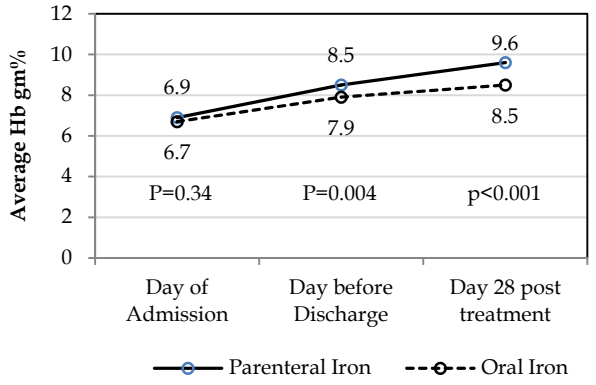

Figure 1 - Comparison of Average Hb on Day of admission, Day before discharge and Day 28 post treatment to the hospital when treated with Oral Iron and Parenteral Iron

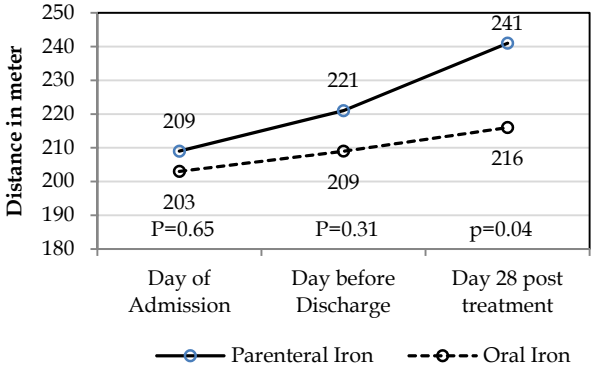

Figure 2 - Comparison of Results of 6-Minute Walk Test on Day of admission, Day before discharge and Day 28 post treatment to the hospital when treated with Oral Iron and Parenteral Iron

Table 2 shows the results of the Iron studies gender-wise which includes serum Iron, serumFerritin, Total Iron binding capacity (TIBC) and percentage saturation of Transferrin (TSAT). Average serum Ferritin value was 17±7.05 ng/ml which was low and it's a sensitive indicator of depletion of body iron stores. Serum Iron values were also low (44.9 ± 31.2 µg/dL).Bone marrow aspiration and microscopic examination was carried out for 72 of the 75 study subjects. Three study subjects did not give consent for the same. A total of 50 patients (69.45%) had erythroid hyperplasia with micronormoblastic pattern of maturation with marrow iron stain of 0 to +1 (absent or depleted bone marrow iron stores). A total of 22 out of 72 (30.55%) study subjects showed erythroid hyperplasia with dimorphic pattern of maturation with iron stain of +2 to +3 (normal bone marrow iron stores or functional IDA). Of the 75 patients in total, 55 received parenteral iron and 20 received oral iron supplements.

Figure 1show the comparison of average hemoglobin on the day of admission, the day before discharge and at 28th day follow-up post-treatment between the two treatment subgroups (parenteral iron and oral iron) using Welch's t-testwith corresponding p-values. Figure 2 shows a similar comparison of results of SMW test on the day of admission, the day before discharge and at 28th day follow-up post-treatment subgroups (parenteral iron and oral iron) using Welch's t-test with corresponding p-values.Table 3 compares the average Hb andresult of SMW test carried out on the day of admission to the hospital and then after 28 days post-treatment for each type of therapy (oral and parenteral) separately.

DISCUSSION

Iron metabolism is tightly regulated to prevent its overload or excess. The mechanism by which iron enters the body is from food or from medicinal iron intake. There is no regulated excretory pathway for iron and the only mechanisms by which iron is lost from the body are blood loss and the loss of epithelial cells from the skin and gut. Non-vegetarian food is a good source of heme iron which is most readily absorbed. On the other hand, vegetarian sources most commonly contain non-heme iron which is not absorbed easily. Non-heme iron contributes about 90-95% of total daily iron in Indian diets.[14] Infants, adolescent girls and adult females are the most vulnerable groups to have iron deficiency since there is an imbalance between the demand and the supply of iron.[14,15,16] Hookworm infestation, heavy menstrual blood loss, pure vegetarian diet, multiple and frequent pregnancies, malaria epidemics are added risk factors for high prevalence of severe IDA in developing countries such as India, Nepal, etc.[14,17]

According to the WHO definition, all study subjects enrolled for the study had severe anemia (Hb< 8 g/dL) on the day of admission to the hospital. A total of 75 patients whose peripheral smear showed a hypochromic microcytic picture with low RBC indices were presumed to have IDA and were further investigated. Our study population was a mix of both genders. Until now, many studies have been done to evaluate the prevalence of IDA during pregnancy and in females of reproductive age group.[18,19,20] But we also included male patients in the study to assess the true prevalence of IDA in the general population. The average age of the study subjects was 40 ± 14.8 years. Average age of study population was 25 years in a similar study of rural Indian patients done by Alvarez-Uria et al. in 2014. [21]This meant that the study subjects were in their prime working age. The nation suffered a productivity loss in terms of valuable person-hours of effort lost due to the subjects being unable to work due to severe anemia. Average RDW in our patients was higher than normal. RDW measures the degree of anisocytosis (size difference) of the population of red cells, and its elevation is neither sensitive nor specific for IDA,[22] RDW is a parameter often used todifferentiate IDA from thalassaemia where RDW is normalalthough as per studies done by AlFadhli et al, Beyan et al and Demir et al, RDW has a low sensitivity and high specificity when used to differentiate between IDA and ß-thalassemia[23,24,25]. The average RPI in our study was 0.3, i.e. it was low, which showed that the bone marrow response to low Hb was inadequate (defective erythrocyte production).[26]This may be either due to a primary bone-marrow disease or secondary to iron deficiency. Iron studies and bone-marrow examination for stainable ironfurther helped in reaching the diagnosis.

Unexplained IDA in hospitalized patients is often a diagnostic dilemma and requires lot of laboratory investigations. It is difficult to differentiate anemia due to iron deficiency from that due to chronic disease / inflammation or malignancy by routine laboratory parameters. By definition, IDA is diagnosed when the iron stores in the body are completely depleted. Absence of stainable iron in the bone marrow has been the gold standard for diagnosis of IDA.[27]But every time a bone marrow sample may not be available for diagnosis. Hence, a diagnostic criterion for IDA has to be based on the indirect measurements of the iron stores. This is done by using conventional lab tests iron status, like RBC indices, serum iron, ferritin, transferrin, transferrin saturation and TIBC. In our study, the average RBC indices, especially MCV (cutoff was < 75fL),werelower than the normal and the TIBC was

in a range of normal to highwhich was similar to the findings of the studies done by Yip et al., Vendt N et al.[28,29]. Low RBC indices may occur in IDA as well as anemia of chronic disease. High level of transferrin receptor in the serum can be used to diagnose IDA[30,31] however, it does not offer any advantage over TIBC for discriminating between people with biochemically defined IDA or ACDas studied by Wians FH et al. [32] Low serum ferritin level is the most sensitive marker for IDA but it lacks specificity as its level is often affected by acute phase responses of the body. A recent study done by Punnonen K et al. has shown that the calculation of the TfR/log ferritin ratio (the TfR-F Index) is a very good indicator of iron depletion. This parameter consists of two variables, influenced by the body iron stores, the availability of iron for erythropoiesis and the total mass of erythroid bone marrow thus, providing highest sensitivity and specificity.[33]

Our study had nearly 70% patients with an absolute iron deficiency (depleted iron stores) on bone marrow examination. A study done by Bell JD et al.on patients undergoing maintenance hemodialysis showed similar findings.[34] The rest had low to normal iron stores suggesting a functional iron deficiency which is defined asan imbalance between the iron demand and supply of the erythroid marrow, thus not allowing the normal hemoglobinization of the erythrocytesas studied by Thomas C et al. [35]

Treatment options for IDA include blood transfusion, parenteral iron and oral iron. Blood transfusion is reserved only for patients in whom there is acute and ongoing blood loss or for those with compromised hemodynamic status. Traditionally, oral iron supplements are safe, effective and cheaper option. However, oral iron use is often limited by gastrointestinal side effects, such as abdominal discomfort, nausea, vomiting, constipation, and dark colored stools.[36] Enteric-coated and delayed release iron supplements have fewer gastricside effects but they are not as well absorbed as the nonenteric-coated preparations.[37] Also, the gut is able to absorb only a limited amount of iron and if the iron requirement exceeds it, then oral iron is unable to replenish the iron stores in the body. Therefore, parenteral iron seems to be the preferred option for treating severe IDA. Safety and efficacy of parenteral iron has been proven through many studies.[38-42]

We observed that compared to oral iron, the parenteral iron therapy when used in treating chronic severe IDA, led to a statistically significant rise in Hb levels at one and four weeks post treatment (p value < 0.05). Similar superior results were seen in other studies done by Kulnigg S et al., Qunibi WY et al. and Van Wyck DB etal.with parenteral ferric carboxymaltosein inflammatory bowel disease patients, in non-dialysis dependent chronic kidney disease patients and in pregnant patients with IDArespectively.[40-42]

A six-minute walk test results showed significant improvement in the functional capacity of the subjects treated with injectable iron as compared to oral iron at 4 weeks of therapy. However, there was no significant difference immediately within one week of the treatment between the oral and injectable iron. Thus, the two primary endpoints of the study, i.e rise in Hb levels and improvement in functional capacity were achieved in a statistically significantly higher manner at four weeks post-treatment in injectable iron group as compared to the oral iron group. This finding was similar to the FAIR-HF trial done by Anker SD et al. on chronic heart failure patients with iron deficiency.[43,44]

On further evaluation, we found that at day 28, among both the orally and parenterally treated subgroups, the Hb level improved in a statistically significant manner as compared to day 1 of admission. But the SMW test results showed that there was a statistically significant improvement only in the parenteral iron subgroup. Thus, both oral and parenteral therapies successfully treat IDA, but with parenteral preparation there is improvement in both the Hb level as well as the functional capacity as evaluated by the SMW test, whereas with oral iron preparation, the functional capacity does not improve significantly.

Ours was an observational study only, thus, we did not decide the treatment protocol for the study subjects. Theother limitation of this study was that the improvement in the iron stores in the bone marrow and other body iron estimation parameters were not assessed at the end of the study period. Whether the findings of the current study can be applied to special populations such as pregnant females, cardiac failure and chronic kidney disease patients is not known as they were excluded from this study. Further studies with longer duration and detailed investigations post-treatment are required to unequivocally prove the superiority of intravenous iron preparations over oral iron replacement.

CONCLUSION

Serum ferritin and bone marrow iron stores estimation together provide a sensitive measure for diagnosing IDA. Parenteral iron preparations are more effective than oral iron therapy in the hospitalized patients with chronic severe IDA. Superiority of parenteral iron over oral iron was demonstrated in the current study by rapid and signifi-

cant improvement in the hemoglobin levels as well as the functional capacity of the patients. Increasing their use may help in reducing the inappropriate over-usage of blood transfusion in hospitalized patients, thus saving precious blood products for those who truly need them.

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