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Utility of low hemoglobin density, intensive method of assessment and classification of bone marrow iron status in patients with dimorphic anemia

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ABSTRACT

Background : The increasing prevalence of combined nutritional deficiencies and co-morbidities has made usage of biochemical markers like serum ferritin, challenging in diagnosing cases of functional iron deficiency. Microscopic evaluation is the "gold standard" method for assessment of iron stores. In this study, we tried to distinction of iron store deficiency from functional iron deficiency and correlate the marrow iron stores with LHD%(Low hemoglobin density). **Aim**: To perform an intensive bone marrow iron grading by assessing iron in fragments, in macrophages around fragments and in erythroblasts and to correlate the marrow iron store results with LHD%. **Materials and Methods**: A descriptive study of Perl's Prussian blue stained bone marrow aspirate smears of 62 adult patients with dimorphic anemia. Bone marrow iron was assessed by both the Gale's method and the intensive method and correlated with LHD%. **Results**: The Conventional Gale's grading revealed hypoferremic state in 32.26% cases and normal iron stores in 56.45% cases. The new Intensive method of grading, showed that most common was functional iron deficiency(35.48%), followed by normal stores, combined deficiency (functional & iron stores) and lastly iron stores deficiency. According to intensive grading method, most of the cases predominantly showed functional iron deficiency. LHD% was statistically significant with the groups obtained from intensive grading system. **Conclusion**: Intensive method of assessment of iron stores helps in identifying patients with functional iron deficiency. LHD% could be an easily available parameter to identify patients with iron deficiency.

Keywords: *Bone marrow iron, Gale's method, intensive method, low hemoglobin density*

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1. INTRODUCTION

Anemia is the most common hematological disorder in all age groups. Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population.[1] Nutritional anemia, particularly iron deficiency and megaloblastic anemia, continues to be a major public health problem worldwide, particularly in the developing countries.[1]Dimorphic anemia has a complex pathogenesis with involvement of more than one deficiency state, usually due to deficiency of both iron and vitamin B12 or folic acid. [2] This condition may be reflected by increased RDW in the presence of normal MCV, with dimorphic blood picture, showing two RBC population, that is combination of microcytic hypochromic and macrocytic normochromic cells. [3] This should be further investigated to rule out iron deficiency and anemia of chronic disease.

The serum iron markers like serum ferritin reflects the total body iron stores and it is also an acute phase response protein, so the concentration of ferritin increases during infection, inflammation, liver disease, malignancy and hemodialysis.[4]These markers, may not discriminate between depleted iron stores and conditions associated with defective reticuloendothelial release of iron (functional iron deficiency).[5,6] Assessment of iron stores in bone marrow aspirate is the gold standard.[7] The conventional Gale method detects only true deficiency of storage iron without any information of functional iron deficiency. However, intensive iron grading assesses iron in erythroblasts, macrophages along with bone marrow fragments.[6] It is important to know about the functional iron deficiency so as to prevent the patient from unnecessary iron

treatment and toxicity.

This study used Gale's method of grading system and intensive grading system to distinguish the functional iron deficiency from iron stores deficiency. Secondly, LHD (Low hemoglobin density)% which is an indirect marker of iron restricted erythropoiesis is included to correlate it with bone marrow iron stores.

2. MATERIALS AND METHODS

This study was conducted on 62 adult patients with Dimorphic anemia and no history of specific therapy or blood transfusion in preceding 4 weeks, at a tertiary care hospital for the period of 1 year. The study was approved by the institution ethics committee. Hemoglobin and RBC indices was estimated using automated cell counter (Beckman Coulter). LHD% (Low Hemoglobin density) was calculated by mathematical transformation of the MCHC value, using the formula used in Beckman coulter instruments.

Bone marrow aspiration was performed on these patients from posterior superior iliac spine after obtaining written informed consent. The procedure was performed under aseptic precautions and material was spread on the slides, smears were air dried and stained with Leishman's stain and Perls' Prussian blue stain. Leishman's stained smears were assessed for Cellularity, M:E ratio, Erythropoiesis, Myelopoiesis and Thrombopoiesis.

Perls' Prussian blue stained smears for the iron stores in bone marrow were first assessed according to Gale's Grading Method[8][Table 1].Smears with atleast seven fragments were assessed, graded and were interpretated as deficient, normal and increased iron stores. Grades 0 and 1, were indicative of iron store deficiency

Table 1: Gale's method of bone marrow iron grading

Grade 0	None	No visible iron under high power magnification (X1000)
Grade 1	Very slight	Small iron particles just visible in few reticulum cells under high power magnification (X1000)
Grade 2	Slight	Small sparsely distributed iron particles just visible under low power magnification (X100)
Grade 3	Moderate	Numerous small iron particles present in reticulum cells throughout the marrow fragment (X100)
Grade 4	Moderate heavy	Larger iron particles throughout the fragment with tendency to aggregate into clumps (X100)
Grade 5	Heavy	Dense large clumps of iron throughout the fragment(X100)
Grade 6	Very heavy	Very large deposits of iron ,both intra- and extra-cellular obscuring cellular details in the fragment (X100)

Perls' stained smears were also assessed using intensive grading method.[6] Fragments, macrophages and erythroblasts were looked for iron particles. Iron particles in the fragments is considered positive only when grade ≥ 2, according to Gale's grading system.

Erythroblasts were considered positive, when more than 30% erythroblasts are showing iron particles. Positive macrophage iron, when iron is present in reticular cells. Results were interpretated as normal iron stores, functional iron deficiency, iron stores deficiency and both functional and iron stores deficiency[6] [Table 2]

Table 2: Iron status category by Intensive grading method.[6]

Fragment	Macrophage	Erythroblast	Iron status category
Present	Present	Present	Normal
Present	Absent	Present	
Present	Present	Absent	Functional iron deficiency
Present	Absent	Absent	
Absent	Present	Present	Iron store deficiency
Absent	Absent	Present	
Absent	Present	Absent	Functional and iron store deficiency
Absent	Absent	Absent	

3. RESULTS

Of the 62 adult patients with dimorphic anemia studied, 33 (53.2%) were male and 29 (46.8%) were female. The age group ranged from 20 to 75 years with mean age of 40.95±17.61 years. Forty nine (79.03%) patients presented with severe anemia (hemoglobin <7 g/dl), 12 (19.35%) patients with moderate anemia (hemoglobin 7-10 g/dl) and one (1.61%) patient with mild anemia (hemoglobin 10-12 g/dl).

Bone marrow examination showed normocellular marrow in 20 (32.36%) cases and hypercellular marrow in 42 (67.74%) cases. None of our cases showed hypocellular marrow.

In our study, Erythroid hyperplasia was seen in 58 (93.54 %) cases , which is indicative of bone marrow response to anemia. The iron status category of these 62 patients assessed by both the Gale's method and intensive method is shown in Table 3

Table 3: Bone marrow iron status category results.

Method	Iron status category	No.	Percentage (%)
Gale's method	Normal	35	56.45
	Increased	7	11.29
	Iron deficiency	20	32.26
Intensive grading method	Normal	20	32.26
	Functional iron deficiency	22	35.48
	Iron store deficiency	5	8.06
	Functional & iron store deficiency	15	24.19

According to Gale's grading system, iron deficient stores was seen in 20 (32.26%) cases, normal stores in 35(56.45%) cases and increased stores in 7 (11.29%) cases. The intensive grading system demonstrated normal marrow iron stores in 20 (32.26%) cases, functional iron deficiency in 22 (35.48%) cases, only iron store deficiency in 5 (8%) cases, both functional and iron store deficiency in 15 (24.19%) cases. According to intensive grading method, most of the cases predominantly showed functional iron deficiency.

Iron status based on LHD%

LHD% is a biomarker of hypochromasia. LHD values were utilized as a predictor of iron status. This value is derived using MCHC values. 10.4 is considered as optimal cut off. LHD% of > 10.4 is used as predictor of iron deficiency[13].

Table 4: LHD % in different iron status categories (Gale's method)

Method - Iron status category	LHD %	
Gale's method	**≤ 10.4**	**>10.4**
Normal	20	15
Increased	3	4
Iron deficiency	1	19

On comparision of LHD% with Gale's method of evaluation of iron stores,15 patients with normal iron stores and 19 patients with iron deficient stores gave increased LHD%. LHD% is strongly significant on comparision of categories obtained from Gale's method of assessing iron stores, with p-value < 0.001, obtained using Fisher exact test.

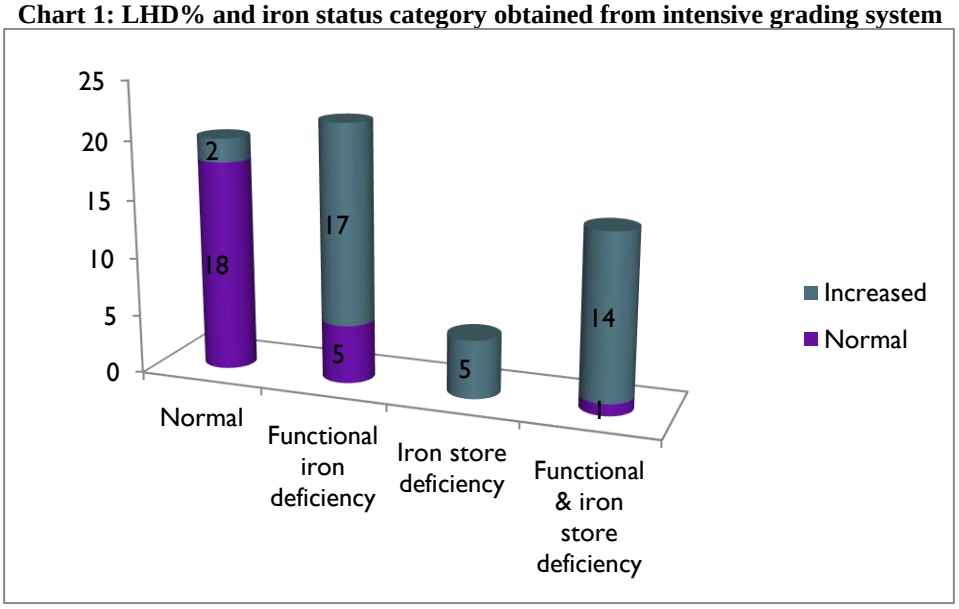
LHD% and intensive grading method for iron stores

LHD% was statistically significant with the groups obtained from intensive grading system and the same is depicted in chart 1. On applying Fisher exact test, LHD% was strongly significant between normal iron stores and functional iron deficiency anemia and also both functional and iron store deficiency, with p-value < 0.001

Table 5: LHD % in different iron status categories (Intensive grading system)

Method - Iron status category	LHD %		Median of LHD %
Intensive grading method	**≤ 10.4**	**>10.4**	
Normal	18	2	2.24
Functional iron deficiency	5	17	33.49
Iron store deficiency	-----	5	32.16
Functional & iron store deficiency	1	14	19.42

Chart 1: LHD% and iron status category obtained from intensive grading system



4. DISCUSSION

Anemia is the most common treatable problem in developing countries.[9] In country like India, most common cause of anemia is nutritional deficiencies. Good proportion of cases show combined deficiency of iron, vitamin B12 and folic acid, where multiple factors affect the diagnostic parameters, resulting in discordant results in bone marrow morphology and iron studies.[7]Megaloblastic anemia is the second commonest nutritional anemia next to iron deficiency anemia in our country.

MCV was normal in 56.45% cases with megaloblastic anemia, could be because of concomitant iron deficiency.[7,10] MCV alone is unreliable as a screening parameter in cases of combined deficiencies.

In uncomplicated megaloblastic anemia, iron stores are normal or increased, both in reticuloendothelial cells and erythroblasts. Ineffective erythropoiesis with increased intramedullary cell death and also peripheral red cell destruction because of their defective membrane architecture, makes the assessment of true iron stores difficult resulting in discordant results.

Assessment of iron stores in bone marrow is considered as "Gold standard" test[7]. The Gale's method of grading system evaluates storage iron in marrow fragment alone[6]. Only the presence of stainable iron does not define the quantity resolubilized and incorporated iron into the developing erythron. Thus, it is important to differentiate iron deficiency anemia from functional iron deficiency. Functional iron deficiency is a state in which there is insufficient incorporation of iron into erythroid precursors in the face of apparently adequate body iron stores.[10] Fragment and macrophage iron reflect iron stores while iron in the erythroblast is indicative of utilizable iron which is diminished in functional iron deficiency.[15] Phiri et al.[6] employed an intensive method of assessing marrow iron in 303 children (aged 6 to 60 months) assessing iron in marrow fragments, in macrophages around fragments, and in erythroblasts. Bableshwar et al.[11] employed an intensive method of assessing iron stores in 80 adult patients. Singh et al.[16] assessed iron stores by intensive method of assessment in 143 adult patients.

Our study indicates that most of the cases with normal, decreased or increased iron stores on Gale's method, predominantly showed functional iron deficiency on intensive method. This may be attributed to ineffective erythropoiesis seen in megaloblastic anemia, which is responsible for poor incorporation of iron into red cells resulting in functional iron deficiency.[10]This findings of bone marrow iron grading were similar to findings pointed out by other studies. Phiri et al[6] and Bableshwar et al[11] found functional iron deficiency as the commonest finding.

Studies on LHD% have proved this as a reliable parameter for study of iron status. LHD% is an hypochromic biomarker. It is an indirect marker of iron restricted erythropoiesis and iron availability in the clinical settings influenced by inflammation and acute phase response.[13] Damodhar et al[13] studied, LHD% with the serum iron markers and concluded that it can be used in the absence of iron profile, as an useful predictor of iron deficiency. In our study, LHD% was correlated with bone marrow iron stores and was statistically significant in iron deficient states. LHD% can be used in diagnosis and monitoring the response to therapy in a reasonable manner.[12,13,14]

In cases of combined nutritional deficiencies of iron, vitamin B12 and folic acid, peripheral blood examination, bone marrow examination and serum ferritin levels may not be of much use for definitive diagnosis, as morphological features of vitamin B12 and/or folic acid deficiencies are masked by iron deficiency. In such cases, intensive method of assessment of iron stores and LHD% may be utilised in providing cost effective health services, by reducing unnecessary iron therapy and repeated hospital admissions.

5. CONCLUSION

Concomitant deficiencies of vitamin B12 and/ or folate along with iron deficiency are not infrequent, one type of anemia may mask the other. Traditional parameters like MCV and serum ferritin provide limited diagnostic value in this setting. Perl's method for grading of bone marrow iron is still regarded as the "Gold Standard". Grading of bone marrow iron stores can be improved by intensive method of assessment over conventional Gale's method and can be used as an important tool to differentiate between true iron deficiency from functional iron deficiency. This could be of high clinical importance in areas where concomitant nutritional deficiencies and inflammatory conditions co-existing with iron deficiency anemia are high.

LHD% could provide added value in identifying iron deficiency. Literature data suggest that LHD% is more sensitive than ferritin, serum iron and transferrin saturation. Which can be determined as part of routine laboratory tests at no extra cost and without additional blood collection, and thus rapid, accurate and convenient additional diagnostic tool for Iron deficiency.

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