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# UTILITY OF LOW HEMOGLOBIN DENSITY AND MICROCYTIC ANEMIA FACTOR IN IRON DEFICIENCY ANEMIA

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## ABSTRACT

**Introduction:** Iron deficiency anemia (IDA) is the most common form of nutritional anemia. Beckman coulter has proposed newer RBC parameters: low hemoglobin density (LHD %) and microcytic anemia factor (MAF) , which can be obtained by calculation and might replace the routine biochemical parameters used for diagnosis of IDA. As these biochemical parameters give discordant results in combined deficiencies and conditions associated with other co-morbidities. We aimed to evaluate the significance of the newer parameters (LHD and MAF) in the diagnosis of IDA and compared them with routinely used biochemical parameters in the diagnosis of IDA

**Methods:** Hundred cases with anemia were included in the study. Hemoglobin levels and RBC indices were obtained from analyser. LHD and MAF were calculated by the formula proposed by Beckman Coulter. Serum iron, TIBC, UIBC and transferrin saturation were obtained by immunoturbidometric assay.

**Results:** The differences of all the parameters between the groups, based on MCV and serum iron were statistically significant. LHD shows negative correlation and MAF shows positive correlation with Hemoglobin, RBC indices, serum iron and transferrin saturation respectively.

**Conclusion:** MAF and LHD% could be used as a screening tool for IDA. These parameters can be obtained as routine blood counts.

**Keywords:** Low hemoglobin density, Microcytic anemia factor, Iron deficiency anemia.

## INTRODUCTION

Iron deficiency anemia (IDA) is the most common form of nutritional anemia worldwide.[1]Iron deficiency anemia is the commonest cause of anemia in the developing countries. RBC indices along with biochemical parameters such as serum iron levels, transferrin, transferrin saturation and ferritin, are widely used to assess iron status and diagnosis of iron deficiency anemia.[1-2]Serum Ferritin and iron both show acute phase responses to inflammation, so iron may fall and ferritin may rise independent of the marrow iron store.[3] These parameters may be less reliable in cases associated with comorbities along with IDA. These biochemical parameters are expensive.

Few of the newer devices includes newer parameters like low hemoglobin density (LHD) and microcytic anemia factor (MAF), which are obtained from calculation using MCHC, MCV and Hb concentration.

LHD is derived from the mean cell hemoglobin concentration (MCHC) and is calculated by using a mathematical sigmoid transformation.[3,4] MCHC is an estimation of both the availability of iron over the preceding 90-120 days and of the proper introduction of iron into intracellular hemoglobin. In the same way, LHD is related to iron availability and the hemoglobinization of the mature erythrocytes.[4,5] Microcytic anemia factor (MAF) is a parameter for examining abnormal red cell modalities, as its calculation accounts for both size and hemoglobin content.[5,6] These parameters require neither extra blood sampling nor extra cost. Urrechaga et al[4,5]. has concentrated on these parameters and conducted many studies but there is still need for more studies. In this study, we aimed to evaluate the significance of newer parameters (LHD and MAF) in the diagnosis of IDA and compared them with routinely used biochemical parameters in diagnosis of IDA.

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**MATERIALS AND METHODS**:

Our study included 100 patients with anemia over a period of 4 months, after the approval from Ethics committee. Anemia was defined as Hb concentration<12 gm/dl for women and Hb concentration <13 gm/dl for men. Blood sample was collected in plain and EDTA vacutainers, serum was separated immediately from plain vacutainers and serum iron, transferrin receptor, transferrin saturation, TIBC and UIBC were determined by immunoturbidimetric assay. EDTA samples were run on the Beckman coulter, to obtain Hemoglobin concentration, RBC counts and RBC indices. This data was analysed and LHD% and MAF were derived from MCHC, MCV and Hb concentration using the formula described by Urrechaga[5]. LHD is derived from MCHC and calculated using the mathematical sigmoid transformation formula of LHD= 100 X √1-{1/(1 + e 1.8 (30-MCHC))}. The MAF is calculated using Hb concentration and MCV, this accounts for both size and hemoglobin content: MAF={(Hb X MCV)/100}.

Patients were considered to be iron deficient if the following criteria were fulfilled—serum iron less than 35 ug/dl, transferrin saturation less than 20 %, mean corpuscular volume (MCV) less than 70 fl. Patients with an MCV more than 70 and anemia were analyzed as a separate group.

**RESULTS**

The 100 patients with anemia were divided into three groups and analyzed. 56 patients were female and 44 were male. The age group ranged from 3 months to 94 years. Eleven cases had mild anemia (> 10 gm/dl), 21 had moderate anemia (8-10 gm/dl) and 68 showed severe anemia (> 8 gm/dl). These 100 cases were divided into two groups based on MCV and analyzed. The data from these two groups is depicted in Table 1

**Table 1: Comparison of iron status based on MCV**

| | **Group 1 MCV less than 70** | **Group 2 MCV more than 70** | **P value** |
|---|---|---|---|
| Number | 63 | 37 | |
| Hemoglobin | 6.11(2-10.3) | 7.79(2.5-13) | 0.0004 |
| RBC | 3.75(1.66-5.33) | 2.8 (0.86-5.6) | 0.0001 |
| MCV | 56.6 (46-70) | 90.32 (72-122) | 0.0001 |
| MCH | 16.256 (11.3-23) | 29.9 (21.9-43.6) | 0.0001 |
| MCHC | 28.29 (24-39.4) | 32.77(13.3-38.4) | 0.0001 |
| Serum Iron | 30.06 (6-182) | 111.9 (10- 337) | 0.0001 |
| Transferrin receptor | 290.72 (56.7-479) | 191.03 (28.9- 317.1) | 0.0001 |
| Transferrin saturation | 14.38(1.57-397) | 44.63(2.2-244.8) | 0.0016 |
| TIBC | 416.39 (81-684) | 275.7 (148-453) | 0.0001 |
| UIBC | 383.14 (63-655) | 169.46 (4-443) | 0.0001 |
| LHD% | 84.69 (0.02-99.9) | 14.9 (0.05-100) | 0.0001 |
| Maf | 3.5 (1-6.93) | 6.79(2.15-10) | 0.0001 |

**Table 2: Patients with anemia with MCV less than 70 and serum iron less than 35 ug/dl**

| | **Group 3** |
|---|---|
| Number | 50 |
| Hb | 6.02 (2.5-8.9) |
| RBC | 3.75(1.77-5.33) |
| MCV | 55.22 (46-66) |

| | |
|---|---|
| MCH | 15.64 (11.3-22) |
| MCHC | 28.10 (24-39.4) |
| Serum iron | 13.87 (6-29) |
| Transferrin receptor | 290.81 (56.7-479) |
| Transferrin saturation | 12.10 (1.57-397) |
| TIBC | 416.85 (81 to 684) |
| UIBC | 400 (63-655) |
| LHD% | 87.86 (0.02-99.9) |
| MAF | 3.37 (1.27-5.42) |

Table1 shows the results of patients with anemia using an MCV less than 70 fl as cut off. Group1 consists of 63 patients with MCV less than 70. Group 1 showed a mean iron of 30.06 ug/dl (range 6-182), mean transferrin saturation of 14.38% (range 1.57-397), LHD% of 84.69 and MAF of 3.5. Group 2 consists of 37 patients with MCV greater than 70 fl. Group 2 showed a mean iron of 111.9 ug/dl (range 10- 337), mean transferrin saturation of 44.63% (range 2.2-244.8), LHD% of 14.9 and MAF of 6.79. The difference in all the parameters was statistically significant. The patients in group 1 were sub-analysed and found that 50 patients had a total iron less than 35ug/dl. The data on these patients is depicted in Table 2, with mean iron of 13.87 ug/dl (range 6-29), mean transferrin saturation of 12.10% (range 1.57-397), LHD% of 87.86 and MAF of 3.5.

MAF showed a positive correlation with hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), serum iron, transferrin saturation and a negative correlation with transferrin and total iron binding capacity. Low hemoglobin density (LHD) showed a negative correlation with hemoglobin, MCV, MCH, MCHC, serum iron, transferrin saturation and a positive correlation with transferrin and total iron binding capacity.

**DISCUSSION**

Many biochemical parameters are being used for the diagnosis of IDA, but most of these parameters are influenced by inflammation, due to the acute phase response. Technological advances in automated complete blood count analyzers have included few newer calculative parameters which enable to show iron status of patients. Beckman-Coulter has proposed LHD% and MAF as potential markers for iron deficiency.[4,6]

Diagnostic sensitivity, specifity and efficiency of LHD% was found to be better when compared to MAF. LHD% (Low hemoglobin density) is derived from the MCHC and calculated using the mathematical sigmoid transformation formula. In a study by Urrechaga, reported the LHD% as a potential indicator of available iron for erythropoiesis.[4]In our study, the LHD% values obtained in Group 1(MCV<70fl) and Group 3 (MCV < 70 fl and serum iron <35 ug/dl) were statistically higher than the Group 2 (MCV >70 fl).

Studies on LHD% have proved this as a reliable parameter for study of iron status. LHD% is a 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.[4,6,7]Damodhar et al[7] 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 compared with routinely used biochemical parameters. LHD% was correlated with serum iron levels and was statistically significant. Hence, LHD% can be used in diagnosis and monitoring the response to therapy in a reasonable manner.[4,5,6]

Microcytic anemia factor (MAF) is a parameter for examining abnormal red cell modalities, as its calculation account for both size and hemoglobin content. Dopsaj et al.[8] reported that MAF performs very well in discriminating different stages of iron deficiency. MAF monitoring is justified as a low cost, effective screening parameter of determining iron status. In our study, mean MAF in a patient with MCV< 70 is 3.5, group with MCV > 70 is 6.79 and in a group with MCV <70 with Serum iron <35 ug/dl is 3.37, MAF values

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were lower in MCV <70 with Serum iron <35 ug/dl than patients with MCV > 70, and is statistically significant. MAF showed positive correlation with hemoglobin, hematocrit, erythrocyte indices, serum iron and transferrin saturation. LHD% showed negative correlation with hemoglobin, erythrocyte indices, serum iron and transferrin saturation. But, many more studies to be done to establish an optimal cut off value and discriminate IDA from other causes of anemia in routine clinical practice. Karagülle Met al.[9] studied on cases with IDA and found that diagnostic sensitivity, specificity and efficiency of MAF was better when compared to LHD.

## CONCLUSION

Our study in concordance with other studies suggest that, in the absence of iron profile for the patients with suspected with iron deficiency anemia, the LHD% and MAF values which can be calculated from the instrument is a useful predictor of iron deficiency. The LHD% is derived from the MCHC while MAF is derived from MCV and Hemoglobin concentration are measured in the instrument and easily incorporated into the report obtained from the instrument as an hemogram. In developing countries like India where iron deficiency is most common anemia and a major problem among children and women, these newer parameters can be obtained with no additional cost. This information will be extremely useful to the primary care physician in diagnosis and monitoring the response to therapy in IDA. This might avoid the repeated request for routine biochemical parameters.

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