Open Access Original Article

Evaluation of Haematological Markers (Microcytic Anaemia Factor and Low Haemoglobin Density) To Distinguish Between Iron Deficiency Anaemia and Thalassemia Trait

Fatima Khurshid[1]
Noor ul ain Yamin[2]
Zainab Kamran[3], M. Usman Khan[4], Jawad Abdul Sattar[5]

[1]KAM School of Life Sciences, Forman Christian College, Lahore, Pakistan .[2-5]Department of Medical Laboratory Technology, Hussain College of Health Sciences, Lahore Pakistan

Address for Correspondence
Fatima Khurshid
KAM School of Life Sciences, Forman Christian College, Lahore, Pakistan.
fatima.khurshid43@gmail.com

Abstract

Objective: To evaluate the diagnostic utility of two hematological markers—microcytic anemia factor (MAF) and low hemoglobin density (LHD) in distinguishing between iron deficiency anemia (IDA) and thalassemia trait (TT), which share similar clinical presentations but require different management strategies.

Methodology: A cross-sectional study, was conducted at Mayo Hospital, Lahore, on 357 patients of which, 76 were healthy control, 90 patients of thalassemia trait and 181 patients of IDA who presented with these diseases between May 2024 and September 2024. The recorded laboratory data of patients include complete blood count, serum ferritin, and Hb electrophoresis. LHD and MAF were calculated from these parameters.

Results: Among the studied patients, 82.4% were females, and 17.6% were males. The age group most significantly impacted by iron deficiency anemia was adults aged 21 to 40 years. Individuals aged 1 to 20 years were most frequently affected by the Thalassemia Trait, and adults aged 61 to 80 years were the least affected by both conditions. LHD (low hemoglobin density) and MAF (microcytic anemia factor) are both statistically significant predictors of IDA. Based on these factors, the whole model is statistically significant ($p < 0.001$) in predicting the presence of IDA.

Conclusion: Higher values of MAF and LHD are associated with lower odds of having IDA. Therefore, these findings suggest that MAF and LHD are important predictors in distinguishing between IDA and absence of IDA.

Keywords: Iron deficiency anemia, Thalassemia trait, Low hemoglobin density, Microcytic anemia factor, Mean corpuscular hemoglobin concentration.

Introduction

Iron deficiency anemia (IDA) has reached epidemic extents in developing nations and has become a primary global community health problem.[1, 2] The WHO has classified iron deficiency anemia (IDA), which affects around 30% of the world's population, adult males may also be at risk based on their general health and socioeconomic situation.[3,4] The hereditary illness known as thalassemia is triggered by a decrease in the manufacture of either the alpha or the beta chain of hemoglobin. The component of red blood cells that carries oxygen is known as hemoglobin.[5, 6]

The frequent clinical manifestations of thalassemia trait and iron deficiency anemia include microcytosis and hypochromic. There are occasions when it is quite difficult to distinguish between the morphological results in the IDA and TT. Effective differentiation between TT and IDA may be achieved by utilizing a battery of assays, such as serum ferritin, and calculation of HbA2 level. It is crucial to distinguish between IDA and TT for two main reasons. Firstly, if the attending physician prescribes excessive iron and misdiagnoses Hb as IDA, the patient's condition won't improve in TT. The second significant concern arises when a person with Thalassemia Trait (TT), who was misdiagnosed as having Iron Deficiency Anaemia (IDA), marries another TT individual, potentially leading to offspring who are homozygous or predominantly affected by thalassemia, concentrated on people who have been diagnosed with TT or IDA but are anemic.[7-9]

The sole sign of thalassemia minor or trait is mild anemia that is not treatable with medication. RBC morphology is very much similar to that of iron deficiency anemia, with a few notable exceptions: nucleated RBCs are uncommon, electrophoretic mobility is normal, and hemoglobin is resistant to alkali. The automated blood cell counts can be used to compute derived indices, such as the RDW

Authorship Contribution: [1] Conceived and planned the idea of the study, final approval of the version to be published, 2Collecting the data, [3]Manuscript drafting, analysis, [5]drafting the work or revising it critically for important intellectual content,

Funding Source: none
Conflict of Interest: none
Received: Dec 16, 2024
Accepted: April 15, 2025

index, to distinguish between IDA and TT. RDW, or coefficient of variation of the volume distribution, is a standard statistical parameter that quantifies the average variance in RBC size as determined by the RBC histogram. RDW is the first index to become aberrant in iron insufficiency, according to numerous research studies.[3]

A range of biochemical criteria is employed in the diagnosis process to distinguish between IDA and Thalassemia trait. Automation in whole blood count analysis has made it possible to display the iron status of patients according to certain computations. The microcytic anaemia factor (MAF), which considers both haemoglobin levels and cell size in its calculation, serves as a metric for analyzing abnormal red cell morphology. No additional blood sampling or expenditure is necessary for these metrics.[4] The size and haemoglobin content are both taken into account in the MAF calculation: MAF = (MCV × Hb)/100. The mean corpuscular haemoglobin concentration serves as the basis for calculating LHD, which is determined through a mathematical sigmoid transformation. MCHC provides a comprehensive indication of iron availability over the preceding 90 to 120 days, as well as the appropriate incorporation of iron into intracellular haemoglobin. Likewise, the haemoglobinization of adult erythrocytes and iron availability are associated with low haemoglobin levels (LHD). MCHC serves as the basis for LHD, which is computed using this statistical formula: LHD = 100 × √ (1 - 1/ (1 + e^ (1.8(30 - MCHC)))). The Beckman Coulter DxH800's new RBC parameters offer helpful information that aids in differentiating between Iron deficiency anaemia and Thalassemia trait, which is crucial for medical decision-making and expediting laboratory testing. [10]

The primary objective of this study was to create a discriminative index to distinguish between IDA and TT. The main objective of this study was to create a discriminative index capable of distinguishing between TT and IDA. LHD% and MAF have been suggested by Beckman-Coulter as potential indicators for distinguishing between patients with thalassemia trait and iron deficiency anaemia. Therefore, the two new discriminative index on an automatic haematology analyser utilizing, low haemoglobin density, and microcytic anaemia factor, to effectively differentiate between TT and IDA. The aim of this study was to assess the importance of these two new parameters in the diagnosis of IDA and thalassemia trait.[4]

Methodology

The study was conducted at mayo hospital Lahore. A total of 357patient data of which, 76 were healthy control, 90 patients of thalassemia trait and 191 patients of IDA. The study was approved by the Ethics Committee of Hussain memorial hospital under HCHS/2024/ERC/70.

Venous blood samples of all patients were taken in EDTA and serum vial by a phlebotomist. CBC was performed on the automated Beckman Coulter haematology analyzer. This apparatus acted on the principle of electrical impedance and is used to measure complete blood cell count (CBC). Haematological biomarkers (MAF and LHD) were calculated from routine blood tests that were performed as part of the patient's clinical evaluation. MAF was calculated by using both haemoglobin levels and cell size while on the other hand, LHD was calculated by mean cell haemoglobin concentration (MCHC). The new RBC parameters are calculated by given formulas;

MAF= {(Hb ×MCV/100)

LHD = 100√1-{1/ (1+e1.8 (30-MCHC))}

Patients diagnosed with IDA and TT proceeded further. Serum ferritin abnormalities were defined as the elevation or reduction from the mentioned range used to diagnose iron deficiency anaemia; Male: 25-350 ng/ml Female: 13-232 ng/ml. Hb electrophoresis works on the principal of Gel electrophoresis in which the charged particles in a buffer solution separate on the bases of their charge to mass ratio in the presence of electric field and their bands formation are seen under the ultraviolet light. Hb electrophoresis abnormalities were defined as the elevation or reduction from the mentioned range to diagnose Thalassemia trait; HbA (95.0 - 97.0), HbF (0.5 - 1.0), and HbA2 (1.5 - 3.5).

Statistics was performed using the Statistical Package for the Social Sciences Statistics software, version 25. The Kolmogorov-Smirnov test was applied to the laboratory data of patients to assess the normality of data. Demographic and clinical data are expressed as median ± IQR. Kruskal-Wallis test was used to explore the differences of laboratory parameters, LHD and MA

between IDA and TT. Logistic regression was applied to evaluate the role of MAF and LHD which are important predictor in distinguishing between IDA and absence of IDA. A p value less than 0.05 was considered statistically significant.

Results

The study comprised 357 patients, including 76 healthy controls, 90 patients with TT, and 191 patients with IDA who visited the Haematology and Chemical departments at Mayo Hospital as illustrated. All the individuals were confirmed cases of TT and IDA assessed by Hb electrophoresis and serum iron level studies. Among all patients, females were greater in count, more predominant cases of iron deficiency, comprising 181individuals (50.7%) of total study population, while males accounted for 10 patients (2.8%), of total study population as presented in Figure 1

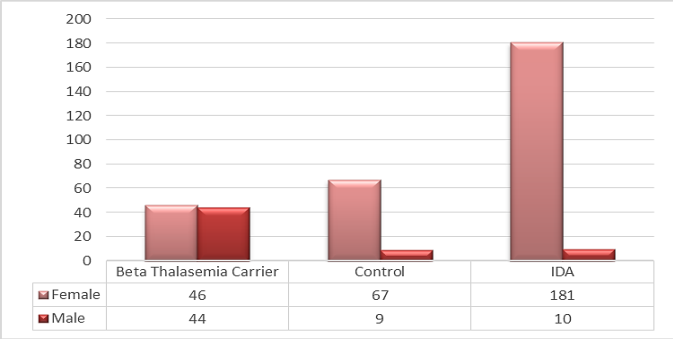

Figure 1 Gender Distribution according to disease

The age group most significantly impacted by iron deficiency anaemia was adults aged 21 to 40 years. Individuals aged 1 to 20 years were most frequently affected by Thalassemia Trait. Contingency table analysis was used to assess disease rates across different age groups. That illustrates the number of cases of both diseases, IDA and TT, along with their corresponding healthy controls. In the 1-20 age groups, beta thalassemia trait showed a higher rate compared to iron deficiency anaemia as in table I.

Since the sample size > 50 Kolmogorov- Smirnov test was applied to assess normality of the Data and the data was not normally distributed so, we proceeded with non-parametric statistical tests.

The table II presents the mean ± standard deviation (SD) of key haematological parameters in individuals with β-thalassemia trait (TT) and iron deficiency anaemia (IDA). Haemoglobin (Hb) levels were lower in IDA (9.3 ± 1.9 g/dL) compared to TT (10.6 ± 1.5 g/dL), indicating more pronounced anaemia in iron-deficient individuals. The RBC count was significantly higher in TT (5.7 ± 0.7 million/μL) than in IDA (4.2 ± 0.6 million/μL), a distinguishing feature of thalassemia trait despite reduced Hb levels. Microcytosis was observed in both conditions, as reflected in the mean corpuscular volume (MCV), which was lower in TT (65.4 ± 6.0 fL) compared to IDA (72.9 ± 9.9 fL). Similarly, the mean corpuscular haemoglobin (MCH) was significantly lower in TT (18.4 ± 2.2 pg) than in IDA (22.2 ± 4.1 pg), reinforcing the more severe hypochromia seen in thalassemia trait. Interestingly, the mean corpuscular haemoglobin concentration (MCHC) was slightly lower in TT (28.3 ± 2.7 g/dL) compared to IDA (30.3 ± 2.2 g/dL), though the difference was less pronounced. These findings highlight key haematological distinctions between IDA and TT. The significantly elevated RBC count in TT, despite reduced Hb, along with a lower MCV and MCH, serves as an essential differentiating factor between the two conditions.

Table I: Number of cases of disease along with controls divided according to age groups.

Age Group	Beta Thalassemia Carrier	IDA	Control
1-20	41	10	15
21-40	39	166	57
41-60	10	10	3
61-80	0	5	1

Table II: Comparison of Haematological Parameters Between β-Thalassemia Trait, Iron Deficiency Anaemia and Controls.

Laboratory Parameters	Thalassemia Trait (Mean ± SD)	Iron Deficiency Anemia (Mean ± SD)	Control (Mean ± SD)
Hb	10.6±1.5	9.3±1.9	9.8±1.6
RBC	5.7±0.7	4.2±0.6	4.2±0.7
MCHC	28.3±2.7	30.3±2.2	30.1±1.7
MCV	65.4±6.0	72.9±9.9	78.5±12.4
MCH	18.4±2.2	22.2±4.1	23.9±4.1

Kruskal-Wallis Test applied on IDA, TT, and Healthy control groups data parameters; Age, Hb, RBC, MCHC, MCV, MCH, Platelet count, HCT, WBC, MAF, LHD. The Kruskal-Wallis test shows significant differences (p-value <0.05) between mean ranks of all three groups (controls, IDA, TT), for age, and other hematological parameters

(Hb, RBC, MCH, MCV, MCHC, HCT, WBC), LHD, and MAF. We found no significant difference for platelets (p-value = 0.894) in all three groups (Controls, TT, IDA). Whereas the groups' mean ranks of Hb, Hct, RBC, MCV, MCH, MCHC, MAF, and LHD values (Control, IDA, and TT) differ statistically significantly from one another. This suggests that the hemoglobin density varies between these groups, which could be a sign of various clinical conditions as presented in table III.

We used Logistic Regression Coefficients for Predicting IDA with MAF and LHD. This indicates that LHD (low hemoglobin density) and MAF (microcytic anemia factor) are both statistically significant predictors of IDA. Higher values of MAF and LHD are linked to lower odds of getting IDA. The odds ratio for MAF was 0.651 ($p < 0.001$), indicating that a higher MAF was associated with a 34.9% reduction in the odds of the outcome. The coefficient for MAF is -0.429, indicating a negative association between MAF and the outcome variable. For every one-unit increase in MAF, the odds of the outcome occurring decrease by 42.9%. The odds ratio is 0.651, which suggests that an increase in MAF reduces the odds of the outcome by approximately 34.9% (1 - 0.651). The Wald statistic is 20.339 with 1 degree of freedom, and the result is statistically significant ($p < 0.001$), meaning that the relationship between MAF and the outcome variable is unlikely to be due to chance.

Similarly, for LHD, the odds ratio was 0.963 ($p < 0.001$), suggesting that higher LHD values were associated with a 3.7% decrease in the odds of the outcome. The coefficient for LHD is -0.038, indicating a smaller negative association between LHD and the outcome variable. For every one-unit increase in LHD, the odds of the outcome occurring decrease by 3.8%. The odds ratio is 0.963, meaning that an increase in LHD reduces the odds of the outcome by 3.7% (1 - 0.963). The Wald statistic is 40.778 with 1 degree of freedom, and this result is also statistically significant ($p < 0.001$), indicating that LHD is a meaningful predictor of the outcome variable.

These results suggest that both MAF and LHD are significant predictors in this model.

Discussion

This study summarizes the role of MAF and LHD in differentiating IDA and TT. According to the research conducted by Jing Lv et al.in his study on evaluating IDA and TT by discriminant factors LHD and MAF concluded that; MAF was higher in TT than in IDA, although LHD was much lower. AUC of 0.9706 (95% CI: 0.9503-0.9909), the cutoff value of 0.5, specificity, sensitivity, positive predictive value, and negative predictive value of 92.91%, 91.36%, 89.16%, and 94.40%, respectively, were the results of the development of a new formula based on LHD and MAF to differentiate between TT and IDA.

Compared to traditional indices like RDW-SD, MCV, and MCH, this novel formula exhibits advantages. Finally, screening for TT and IDA may benefit from the RBC parameters LHD and MAF, which are found using a hematology analyzer. Our novel formula is quick and easy to use, has excellent sensitivity and specificity compared to current discriminant formulas in the literature, and can help with early detection and management.[3, 11, 12]

J Y Zhan et al., 2020 in his study on predictive values of routine blood tests for IDA patients by including LHD and MAF ratios evaluate the results. Blood test results,

Table III: Descriptive Statistics and ranks of disease (TT, IDA), controls by Kruskal-Wallis Test Results.

Variables (N=384)	Mean ± SD	Control (N=137) Mean Rank	Thalassemia (N=99) Mean Rank	IDA (N= 198) Mean Rank	Kruskal-Wallis H	P value
Age	9.77±1.89	157.84	130.99	198.86	30.199	0.000
Hb	4.63±0.97	178.90	220.15	149.68	30.609	0.000
RBC	29.76±2.43	138.12	283.52	133.70	147.468	0.000
MCHC	72.02±10.59	190.47	116.63	194.52	39.927	0.000
MCV	21.5±4.22	228.48	108.81	185.27	59.952	0.000
MCH	329.9±103.5	230.61	94.13	191.46	84.241	0.000
Platelets	27.12±8.09	170.98	170.46	175.79	0.225	0.894
HCT	16.74±13.51	232.73	54.26	209.53	175.868	0.000
WBC	7.10±1.94	125.45	301.16	129.70	198.245	0.000
MAF	60.41±34.39	208.27	171.44	162.64	10.145	0.006
LHD	27.34±11.24	156.53	230.64	152.35	40.318	0.000

including Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), erythrocyte distribution width (RDW), and percentage of low haemoglobin density (LHD), in healthy controls, non-anemic iron deficiency (ID), non-ID anemic, and iron deficiency anemia (IDA) groups were compared using analysis of variance (ANOVA) or non-parametric tests. Quantitative data were described as means ± standard deviation or median (interquartile range), predictive values of routine blood test results and LHD for the detection of IDA and ID were also assessed.[7, 13] Our study also reveals the, significant variations in values of MAF and LHD in TT and IDA patients and statistically proved that haemoglobin density varies between these groups which could be an indicator of other diseases. As it is difficult to evaluate the differences between IDA patients and Thalassemia trait patients by our study and other studies it is cleared that LHD and MAF as well as RDW, MCHC, and MCV are the parameters are the factors helpful in distinguishing these two diseases.

Karima Faragh conducted a study on the utility of low hemoglobin density to detect iron deficiency in IDA patients he mentioned in his study that, Percent LHD has recently been proposed as a possible indicator of available iron for erythropoiesis. LHD% represents the time-averaged value of the degree of haemoglobinization of mature erythrocytes. This analysis can be performed alongside conventional blood tests at no additional cost and without requiring an additional blood sample. MCHC, the parameter from which LHD% is calculated, is a stable laboratory parameter that can be reliably analyzed in samples stored for up to 36 hours." LHD% promises to be a simple, inexpensive, and sensitive tool for accurately and reliably diagnosing iron deficiency with or without anaemia in patients with inflammatory diseases." LHD% is clinically useful not only as a marker of iron-restricted erythropoiesis but also in differentiating between IDA and thalassemia, as reported by Urrechaga et al.[10, 14-16]

Kruskail wallis test used to assess differences between IDA, TT, and Healthy control groups; Age, Hb, RBC, MCHC, MCV, MCH, Platelet count, HCT, WBC, MAF, LHD. We found a significant difference between the means of three groups including the MAF and LHD except the platelets count that were represented by its median ± IQR and P value. LHD (low haemoglobin density) and MAF (microcytic anaemia factor) are both statistically significant predictors of IDA. Based on these factors, the whole model is statistically significant ($p < 0.001$) in predicting a presence of IDA as compared to thalassemia trait. So, in this research we discussed that how MAF and LHD play an important role to differentiate iron deficiency anaemia from thalassemia trait. Further, this study indicated the clinical significance of new Beckman-coulter that contain these two parameters of MAF and LHD.

Previous study just introduced the new parameters of MAF and LHD that play an important role in differentiating iron deficiency anaemia and thalassemia trait while in this study we evaluate and calculate the MAF and LHD through routine blood count and their statistical analysis play an important role to evaluate the main difference between TT and IDA.[10]

Conclusion

The RBC parameters LHD and MAF detected by haematology analyzer could be useful for differentiating TT and IDA. The variable MAF and LHD are statistically significant predictors of IDA. Both variables have negative coefficients, indicating that higher values of MAF and LHD are associated with lower odds of having IDA. MAF and LHD can be used as a cost-effective and reliable biomarker to evaluate the difference between Iron deficiency anaemia and Thalassemia trait.

When diagnosing IDA and MAF, the Beckman-Coulter automated complete blood count instrument offers highly helpful characteristics. Regular blood counts can yield these values without incurring additional costs or requiring further blood sampling. The cut-off values for these factors need to be determined by further research before they can be used in routine practice.

References

1. Kumar SB, Arnipalli SR, Mehta P, Carrau S, Ziouzenkova O. Iron deficiency anemia: efficacy and limitations of nutritional and comprehensive mitigation strategies. Nutrients. 2022 Jul;14(14):2976. https://doi.org/10.3390/nu14142976
2. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann N Y Acad Sci. 2019 Aug;1450(1):15-31. https://doi.org/10.1111/nyas.14092
3. Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harb Perspect Med. 2013 Jul;3(7):a011866. https://doi.org/10.1101/cshperspect.a011866

4. Sharma J, Devanathan S, Sengupta A, Rajeshwari PN. Assessing the prevalence of iron deficiency anemia and risk factors among children and women: a case study of rural Uttar Pradesh. Clin Epidemiol Glob Health. 2024 Mar;26:101545. https://doi.org/10.1016/j.cegh.2024.101545
5. Bajwa H, Basit H. Thalassemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545151/
6. Marengo-Rowe AJ. The thalassemias and related disorders. Proc (Bayl Univ Med Cent). 2007 Jan;20(1):27-31. https://doi.org/10.1080/08998280.2007.11928230
7. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MBD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci. 2017 May;33(3):665-9. https://doi.org/10.12669/pjms.333.12098
8. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. Anemia. 2014;2014:576738. https://doi.org/10.1155/2014/576738
9. Tripathi N, Soni JP, Sharma PK, Verma M. Role of haemogram parameters and RBC indices in screening and diagnosis of beta-thalassemia trait in microcytic, hypochromic Indian children. Int J Hematol Disord. 2015;2(2):43-6.
10. Matos JF, Dusse LM, Borges KB, de Castro RL, Coura-Vital W, Carvalho M. A new index to discriminate between iron deficiency anemia and thalassemia trait. Rev Bras Hematol Hemoter. 2016 Jul-Sep;38(3):214-9. https://doi.org/10.1016/j.bjhh.2016.05.011
11. Lv J, Li J, Ren X, Fan C, Chong H, Huang Q, et al. Differentiation between thalassemia trait and iron deficiency anemia based on low hemoglobin density and microcytic anemia factor. Clin Lab. 2023;69(4). https://doi.org/10.7754/Clin.Lab.2023.230418
12. Saboor M. Discrimination of iron deficiency, alpha and beta thalassemia on the basis of red cell distribution width and reticulocyte indices. Clin Lab. 2021;67(1). https://doi.org/10.7754/Clin.Lab.2020.201008
13. Singh A, Chaudhary R, Pandey H, Sonkar A. Identification of iron status of blood donors by using low hemoglobin density and microcytic anemia factor. Asian J Transfus Sci. 2018 Jan-Jun;12(1):46-50. https://doi.org/10.4103/ajts.AJTS_30_17
14. Martin-Cabrera P, Hung M, Ortmann E, Richards T, Ghosh M, Bottrill F, et al. Clinical use of low haemoglobin density, transferrin saturation, bone marrow morphology, Perl's stain and other plasma markers in the identification of treatable anaemia presenting for cardiac surgery in a prospective cohort study. J Clin Pathol. 2015 Nov;68(11):923-30. https://doi.org/10.1136/jclinpath-2015-203024
15. Urrechaga E, Unceta M, Borque L, Escanero J. Low hemoglobin density potential marker of iron availability. Int J Lab Hematol. 2012 Feb;34(1):47-51. https://doi.org/10.1111/j.1751-553X.2011.01355.x
16. Urrechaga E. The new mature red cell parameter, low haemoglobin density of the Beckman-Coulter LH750: clinical utility in the diagnosis of iron deficiency. Int J Lab Hematol. 2010 Feb;32(1 Pt 1):e144-50. https://doi.org/10.1111/j.1751-553X.2008.01127.x