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Identification of iron status of blood donors by using low hemoglobin density and microcytic anemia factor

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Abstract:

BACKGROUND: Two new parameters low hemoglobin density (LHD) and microcytic anemia factor (Maf) have been used by Beckman–Coulter LH series analyzers as an easy screening tool for the early detection of iron deficiency. The main objective of this study was to assess if LHD and Maf could be used for assessment of iron status in blood donors and also to establish a cut-off for these two parameters at which a tentative iron deficiency could be reported conclusively.

MATERIALS AND METHODS: LHD% and Maf could be calculated by knowing mean cell hemoglobin (Hb) concentration, Hb, and mean cellular volume and we used SPSS in calculating LHD and Maf from these parameters.

RESULTS: Significant differences were detected in LHD% and Maf values when iron deficient and iron-depleted donors were compared with control donors, while these were insignificant for iron reduced donors. LHD and Maf were able to differentiate between iron deficient and iron-depleted donors from normal donors. A cutoff of 9.18% for LHD% was able to differentiate iron deficient and depleted state from normal iron states with a sensitivity and specificity of 91.9% and 71% respectively. Similarly, a cutoff of 10.16 and10.71 for Maf was able to differentiate between iron-deficient and iron-depleted donors from normal donors, respectively.

CONCLUSION: LHD% and Maf in the screening of blood donors raise the possibility of early detection of iron deficiency, without the need of extra cost and blood sampling.

Keywords:

Iron deficiency, low hemoglobin density, microcytic anemia factor

Introduction

Iron deficiency is a major public health problem all over the world, and it is the most common nutritional deficiency in both developed as well as developing countries.[1] Iron plays a central role in erythropoiesis and is required for many physiologic functions, such as cellular respiration, electron transport, and gene regulation. Clinical conditions other than anemia which are associated with iron deficiency are neuropsychological changes, restless leg syndrome (RLS), and pica.[2] Iron deficiency can result from various factors such as reduced intake, impaired absorption, increased requirement, and chronic blood loss.[3] In developing countries like India, inadequate dietary intake is the most common cause of iron deficiency. Repeated blood donation may also lead to iron deficiency.[4] In India, a healthy individual can donate blood at every 3 months provided that before every blood donation hemoglobin (Hb) is 12.5 g/dl or more.[5] After every blood donation, approximately 242 mg and 217 mg of iron are lost with each whole blood donation by male and female donors, respectively.[6] In India, there is no recommendation for screening blood donors for iron deficiency

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even though several studies[7,8] have observed high prevalence of iron deficiency in this population, particularly among females.

Low hemoglobin density (LHD)[9] and microcytic anemia factor (Maf) have been proposed by Beckman–Coulter LH series analyzers as an easy screening tool for the early detection of iron deficiency. The use of LHD and Maf as a screening tool for iron deficiency has been well documented in various groups of individuals including athletes and in some pathological conditions such as congenital heart disease and chronic kidney diseases.[10]

The use of Hb as a screening tool for blood donation has certain limitations in that it cannot differentiate between iron-replete and iron-depleted states. LHD and Maf are easy to calculate using statistical tools, and their use as a screening tool for iron status in blood donors would provide an easy method for identifying blood donors at risk for iron deficiency. The main objective of this study was to assess if LHD and Maf could be used for assessment of iron status in blood donors and also to establish a cutoff for these two parameters at which a tentative iron deficiency could be reported conclusively.

Materials and Methods

This was a prospective study which included blood donors coming to the Department of Transfusion Medicine, in a tertiary care center, North India. Donors were selected every day during the study period by simple random sampling. Donors who were deferred due to low Hb (<12.5 g/dl) were also included in the study. All the donors including the deferred donors were assessed physically and medically as per the regulatory requirements for blood donation in India.[11] Venous blood samples both ethylenediaminetetraacetic acid (EDTA) and plain from the blood donors were then collected for testing after taking their consent. Complete hemogram of all the donors was done by EDTA sample within 4 h using Automated Hematology Analyzer Sysmex KX-21 (Sysmex KX-21, Automated Hematology Analyzer, Sysmex America, Inc.). Serum samples were centrifuged and the serum was stored at −80°C till further testing was done. Serum ferritin (SF) was done on the serum with cobas e 411 (Roche Diagnostics GmbH Germany).

Sample size is calculated by taking the sensitivity of LHD using the formula used for sample size (n) is:[12]

$$n = \frac{z_{\alpha}^{2} S_{n} \left(100 - S_{n}\right)}{pe^{2}}$$

Where S_n = 80%, sensitivity of LHD[13]

P = 5%, prevalence of lack of iron in regular donors.[14]

e = 0.2, error factor

Type I error (level of significance) $\alpha = 0.05$

Data loss fraction = 5%

Then, minimum sample size required to be n = 323.

Blood donors (n = 323) were further divided into four donor groups on the basis of SF.[8,15] Group I: Iron-deficient donors (n = 37), SF was <12 ng/ml. Group II: Iron-depleted donors (n = 10) SF was ≥12–<15. Group III: Iron-reduced donors, SF was ≥15–<30 ng/ml. Group IV (control): Normal or iron-replete donors (n = 248), SF was >30 ng/ml.

LHD% is derived from mean cell hemoglobin concentration (MCHC), using the mathematical sigmoid transformation:[9]

$$\text{LHD}\,\% = 100\sqrt{1 - \left(1 / \left[1 + e^{1.8(30 - \text{MCHC})}\right]\right)}$$

Like MCHC, LHD% tells about the availability of iron over the preceding 90–120 days and hemoglobinization of mature red blood cells (RBCs).

Maf is the product of Hb and mean cellular volume (MCV).[10]

Maf = (Hb × MCV)/100

Statistical analysis

Data were analyzed with Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS, Inc., Chicago, IL, USA). LHD% and Maf were calculated with the help of SPSS. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of LHD% and Maf for the assessment of the iron status of blood donors. Cutoff values were established based on the optimal combination of sensitivity and specificity. Wherever required, data are presented as mean ± standard deviation (SD) for normally distributed parameters and median (25^{th}–75^{th} percentiles) for parameters that were not normally distributed.

$P < 0.05$ was considered statistically significant.

Results

A total of 323 blood donors were included in the study, which included 42 donors deferred due to low Hb as per the donation criteria. These 323 blood donors were divided into four groups based on SF and analyzed. Comparison of Hb, MCV, MCH, MCHC, red cell

Table 1: Hematological parameters in various groups

SF (ng/ml)	Group I (*n*=37) <12	Group II (*n*=10) ≥12-<15	Group III (*n*=28) ≥15-<30	Group IV (*n*=248) ≥30
Hb (g/dl)	10.51±1.34	12.11±0.73	13.67±0.97	14.3±1.3
MCV (fL)	74.37±14.3	83.78±3.79	89.94±7.93	89.19±7.06
MCH (pg)	24±3.76	27.01±1.3	30.64±2.66	29.87±2.97
MCHC (g/dl)	30.08±2	31.47±1.51	33.94±3.8	33.26±2.41
RDW-SD[ǂ] (fL)	48.7 (46.2-50)	49.3 (45.25-52.30)	49.5 (44.6-51)	47.9 (44.1-51)
Maf	7.87±1.95	10.29±0.89	12.29±1.39	12.77±1.64
LHD[ǂ] (%)	67.46 (16.31-95.09)	27.29 (10.52-57.23)	4.13 (2.79-6.71)	6.13 (2.99-12.52)

Data are presented as mean±SD for normally distributed parameters, [ǂ]Parameters that were not normally distributed are shown as median (25th-75th percentiles). SF = Serum ferritin, Hb = Hemoglobin, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, RDW = Red cell distribution width, Maf = Microcytic anemia factor, LHD = Low hemoglobin density; SD = Standard deviation

distribution width-SD, Maf, and LHD% among these is shown in Table 1.

Significant differences were detected in LHD% and Maf values ($P < 0.05$), when iron-deficient (Group I) and iron-depleted (Group II) donors were compared with normal/control donors (Group IV), while these were insignificant for iron-reduced donors (Group III).

ROC curve for LHD% and Maf was done and optimal cutoff and area under curve (ACU) was calculated among iron-deficient, iron-depleted, and iron-reduced states [Figures 1-3]. The optimal cutoff for LHD% was found to be 9.18 which differentiated both iron-deficient donors and iron-depleted donors from the donors with normal iron stores. The sensitivity, specificity, and AUC for iron-deficient and iron-depleted donors were 91.9%, 71%, and 0.902 and 90%, 71%, and 0.856, respectively. Positive predictive value (PPV) and negative predictive value (NPV) of LHD% for iron-deficient states were 32.1% and 98.3%, respectively, while for iron-depleted state 11.1% and 99.4%, respectively [Table 2].

Similarly, the optimal cutoff for Maf was found to be 10.16 and 10.71 which differentiated iron-deficient donors and iron-depleted donors from the donors with normal iron stores. The sensitivity, specificity, and AUC for iron-deficient and iron-depleted donors were 94.6%, 96.3%, 0.981 and 90%, 90.3%, 0.920, respectively.

PPV and NPV of LHD% for iron-deficient donors were 81.4% and 99.2%, respectively, while for iron-depleted donors 27.3% and 99.4%, respectively [Table 2].

Table 2: Statistical data of Group I and Group II

SF (ng/ml)	*P*	Sensitivity	Specificity	PPV	NPV
Group I (*n*=37) <12					
LHD (%)	<0.05	91.9	71	32.1	98.3
Maf	<0.05	94.6	96.8	81.4	99.2
Group II (*n*=10) ≥12-<15					
LHD (%)	<0.05	90	71	11.1	99.4
Maf	<0.05	90	90.3	27.3	99.6

PPV = Positive predictive value, NPV = Negative predictive value, Maf = Microcytic anemia factor, LHD = Low hemoglobin density, SF = Serum ferritin

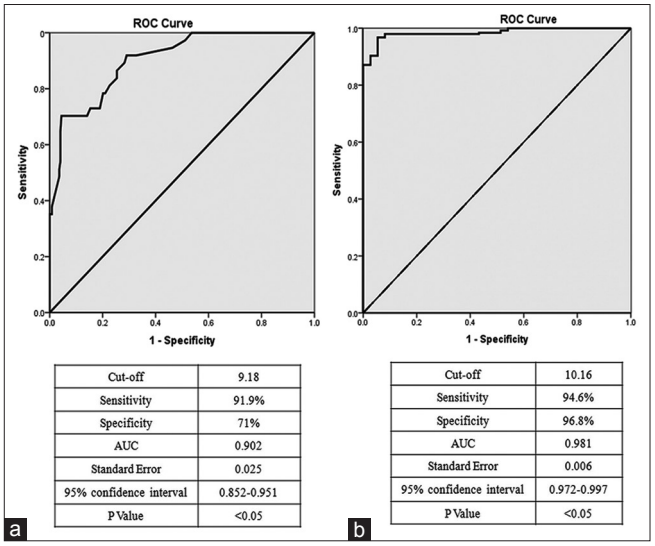
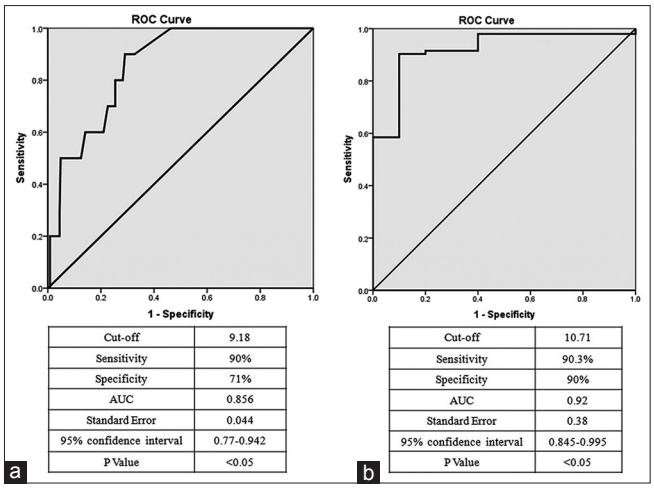


Figure 1: Receiver operating characteristic curve of low hemoglobin density % (a) and microcytic anemia factor (b) to discriminate iron-deficient blood donors from control/normal donors. AUC: area under curve, *P* value: Probability values

Discussion

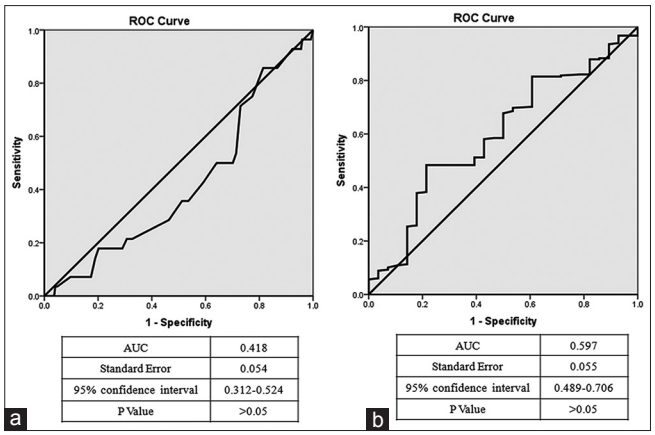
A transfusion medicine specialist has dual responsibility of providing safe blood to the patients as well as to protect the blood donors from any untoward effects of blood donation. Anemia is a common problem among regular blood donors as approximately 242 and 217 mg of iron are lost with each whole-blood donation by male and female donors, respectively,[6] which amounts to nearly 4%–10% of the total body iron.[16] Therefore, maintenance of iron balance is a major challenge in the blood donor population, especially in regular blood donors and female blood donors. The occurrence of iron deficiency in blood donors in India is well documented in the literature.[7,8] Mahida *et al*.[7] reported that among regular voluntary blood donors, 9.5% male and 26.7% female donors developed anemia, while Mittal *et al*.[8] reported that among first-time blood donors, 21% male and 46% female donors had SF <15 ng/ml at the time of presentation.



Cut-off	9.18
Sensitivity	90%
Specificity	71%
AUC	0.856
Standard Error	0.044
95% confidence interval	0.77-0.942
P Value	<0.05

Cut-off	10.71
Sensitivity	90.3%
Specificity	90%
AUC	0.92
Standard Error	0.38
95% confidence interval	0.845-0.995
P Value	<0.05

Figure 2: Receiver operating characteristic curve of low hemoglobin density % (a) and microcytic anemia factor (b) to discriminate iron depleted blood donors from control/normal donors. AUC: area under curve, *P* value: Probability values



AUC	0.418
Standard Error	0.054
95% confidence interval	0.312-0.524
P Value	>0.05

AUC	0.597
Standard Error	0.055
95% confidence interval	0.489-0.706
P Value	>0.05

Figure 3: Receiver operating characteristic curve of low hemoglobin density % (a) and microcytic anemia factor (b) to discriminate iron reduced blood donors from control/normal donors. AUC: area under curve, *P* value: Probability values

The stages of developing iron deficiency are now well understood. Initially, iron stores get depleted which is followed by impairment of red cell synthesis and iron-dependent various enzymes and at last Hb level falls with the development of anemia. At this stage, iron supply becomes rate limiting for red cell synthesis leading to formation of microcytic cells, which dilute the normal red cells already present in circulation.[17]

There are few safeguards to protect the blood donors from developing anemia. Screening of all the blood donors by measurement of Hb and an interdonation interval of 3 months are some of the measures to protect blood donors from anemia. However, these too have certain limitations. Hb levels tend to remain at normal levels until the anemia becomes severe. This is quite evident from our study that ~10% of the donors had Hb levels around 12.5 g/dl, but they had reduced or depleted iron stores (SF <30 ng/ml). Similarly, other red cell indices such as MCV, MCH, and MCHC tend to remain in normal range even though the iron stores are either reduced or depleted. It is thus possible that regular donors and female donors who have normal Hb (i.e., >12.5 g/dl) yet reduced iron stores will end up donating blood.

To overcome this limitation of screening the blood donors with Hb, newer methods to identify donors with reduced iron stores need to be explored. The present study tried to find out if LHD% and Maf could be used to screen blood donors for risk of developing anemia due to low iron stores. These two parameters could be easily calculated using the formula mentioned in Material and Methods. LHD% of 9.18 was able to differentiate between iron-deficient or iron-depleted states from normal iron states, with a sensitivity and specificity of 91.9% and 71%. Similarly, Maf was found to be a good screening tool to differentiate iron-depleted or iron-deficient stores from normal iron stores with a sensitivity and specificity of >90%. The cutoff value of Maf was found to be 10.16 and 10.71 for iron-deficient and iron-depleted states, respectively.

The use of LHD% and Maf to diagnose deficient iron store has been done previously in other group of individuals such as athletes and in some pathological conditions such as congenital heart disease and chronic kidney diseases.[10] This is the first study on blood donors.

This and various other studies show that these two parameters can play an important role in assessment of iron status. A single test cannot access the iron status because iron metabolism is a dynamic process.[9] These parameters may be helpful in the following ways: First, in the assessment of iron status of the blood donors and identifying blood donors at risk of anemia; second, diagnosing iron deficiency in blood donors deferred because of low Hb (i.e., <12.5 g/dl); third, follow-up of regular blood donors; and fourth, for monitoring iron status of blood donors who were started on iron supplements. These parameters are easily obtained by hematology analyzers, without any extra sampling and cost. There were certain limitations of the present study. First, we could not compare the utility of these parameters among male and female donors as the number of female donors presenting to our center is very less. Second, a comparison among repeat donors and first-time donors could not be done due to paucity of repeat donors. However, we were able to describe the utility of these two simple parameters in the setting of blood donation. Further studies are needed to find the cutoff for these parameters among different groups.

Conclusion

Iron deficiency is a potential problem for all blood donors. Laboratory investigations for estimation of iron stores are not feasible for each blood donor. From this study, we concluded that use of LHD% and Maf in the screening of blood donors raises the possibility of early detection of iron deficiency, without the need of extra cost and blood sampling.

Further, a large multicenter study is necessary for the possible clinical use of these two parameters in the screening of blood donors.

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Conflicts of interest

There are no conflicts of interest.

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