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"Evaluation Of Iron Deficiency Anemia By Extended Red Cell Parameters And Its Correlation With Serum Iron,Serum Ferritin ,Transferrin (Saturation) And Total Iron Binding Capacity (Tibc)"

A.Mashila
Sri Ramachandra Institute of Higher Education and Research,Porur- Chennai

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ABSTRACT: By considering the eRBC parameters obtainable from the CBC analyzers, our current study, thus, aims to evaluate the potential utility and reliability of these parameters in distinguishing iron-deficient erythropoiesis related disorders. This study would then give a clearer diagnostic insight to better stratify and classify the iron deficient erythropoiesis related disorders in order to improve treatment plan and patient outcome.

I. INTRODUCTION :

Iron Deficiency is defined as the reduction of iron stores that leads to IDA. IDA is a common condition whereby low levels of iron are associated with anemia and the presence of Microcytic Hypochromic Red cells. In most cases , this deficiency disorders may be diagnosed through complete blood count and low levels of serum ferritin.

Many New Hematologic parameters have become available from modern automated hematology analyzers in recent years . These New parameters include Low Hemoglobin Density (LHD) and Microcytic anemic Factor (MaF).

Automated Hematologic Data Collection :

Data collected from the DxH 800 included the conventional parameters of red blood cells, reticulocytes, and platelets, as well as many newly described hematologic parameters, such as LHD, microcytic anemia factor, mean sphered cell volume, red cell size factor, immature reticulocyte fraction, mean reticulocyte volume, high–light-scatter reticulocytes, reticulocyte distribution width, and mean platelet volume.

Unicel DxH 800 (Beckman Coulter, Miami, Florida,USA) utilized to perform CBC within 6 hours of patient sample collection which is in concordance with the International Council for Standardization in Hematology(ICSH) guideline.

The biochemical parameters highlighted within this study were quantified by the use of assays for serum ferritin (Modular E170, Roche, Switzerland) and soluble transferrin receptor (Cobas Integra 400, Roche, Switzerland). Our laboratory is enrolled in the proficiency programs which covered full blood count, hemoglobin, and serum ferritin analysis. Quality control (QC) had been performed internally to determine precision of assays used while external QC determined accuracy of said assays.

By considering the eRBC parameters obtainable from the CBC analyzers, our current study, thus, aims to evaluate the potential utility and reliability of these parameters in distinguishing iron-deficient erythropoiesis related disorders.

This study would then give a clearer diagnostic insight to better stratify and classify the iron deficient erythropoiesis related disorders in order to improve treatment plan and patient outcome.

AIM OF THE STUDY :

Aim:

To Evaluate the iron deficiency anemia by extended red cells parameters and its correlation with serum iron, serum ferritin, transferring (saturation) and Total Iron Binding Capacity".

Objective:

- To assess the eRBC parameter Maf by the formula

Maf= ((Hb*MCV)/100))

- To assess the eRBC parameter LHD% by the formula LHD%=[100√ 1 1= 1 e1:8(30-MCHC) ½]

II. REVIEW OF LITERATURE :

Angeli Ambayya,Andrew Octavian Sasmita and Jameela Sathar their study is about -Utilizing Extended Red Blood Cell Parameters to Distinguish Iton- Deficient Erythropoiesis – Related Disorders in Malaysian female population

.They study conclusively reported the utility of eRBC parameters to distinguish LID from IDA.(01)

Dopsaj V, Martinovic J,Dopsaj M (2014) Early

detection of iron deficiency in elite athletes: Could microcytic anemia factor (Maf) be useful? This study shows that Maf performs very well in discriminating healthy athletes and those with different stages of iron deficiency.(02)

Int J Lab Hematol. This study is **distinguishing iron deficiency anaemia from thalassaemia trait.** The purpose of this study to evaluate the new red cell parameters on the Beckman Coulter DxH 800 in distinguishing between IDA and TT.(03)

***in* Asian Journal of Transfusion Science** studied about identification of iron status of blood donors by using LHD and MaF. They found two parameters LHD and Maf have been used by Beckman-Coulter LH series analyzer as an easy screening tool for early detection of iron deficiency.(04)

Evaluation of derived coulter red blood cell parameters for the assessment of iron deficiency in adults with congenital heart disease , this study is done by **Dopsaj v , mikovic Golubovic G ,martinovic j,kalimanovska ostrich D** . Aim of they study was to evaluate derived red blood cell parameters in determining the presence of iron depletion and iron deficient erythropoiesis as states that precede IDA in adults with Congenital Heart Disease.(05)

Med Clin North Am. The study about Microcytic anemia that Differential diagnosis and management of iron deficiency anemia. They study says that significant iron loss requires replacement with iron supplements.(06)

Appl Physiol Nutr Metab. Article is about Depleted iron stores and iron deficiency anemia associated with reduced ferritin and hepcidin and elevated soluble transferrin receptors. This study is confirmed that iron deficiency without anemia is more common than iron deficiency with anemia, the correspondingly reduced circulating hepcidin would have enabled heightened absorption of dietary iron in support of erythropoiesis.(07)

International Journal of Clinical and Experimental Pathology . They says that **unusual case of iron deficiency anemia is associated with extremely low level of transferrin receptor**. They demonstrate that this is not a case of systemic iron deficiency, but rather cellular iron deficit due to the low level of transferrin receptor, particularly in erythroid tissue.(08)

IDA study is done by **Lancet.** he studied that several chronic disease are frequently associated with IDA . Notably chronic kidney disease , chronic heart failure , cancer and inflammatory bowel disease.(09)

J Pediatr Hematol Oncol. They determinating the nature of anemia in infection by finding serum Transferrin Receptor levels in children . They studied review that the values are significantly lower in infectious anemia than iron deficiency states.(10)

Clin J Am Soc Nephrol. Study is about assessing iron states beyond serum ferritin and transferring saturation. They reviewed that the increasing prevalence of multiple comorbidities among anemic patients with chronic kidney disease has made the use of serum ferritin and transferrin saturation more challenging in diagnosing iron deficiency.(11)

Natasha M. Archer &Carlo Brugnara they both studied about **diagnosis of Iron Deficient States by Biochemistry and Hematologic analytes.** They examine the most significant hematological and biochemistry markers for iron metabolism as well as relavant genetic polymorphism and defect affecting iron handling.(12)

Nephrol Dial Transplant. They done about **Mature erythrocyte parameters as new markers of functional iron deficiency in haemodialysis (sensitivity and specificity).** They study to assess the sensitivity and specificity of mature erythrocyte parameters in detecting functional iron deficiency (FID).(13)

Arch Argent Pediatr. His objective is to evaluate the **efficacy of soluble transferrin receptor (sTfR) in diagnosing iron deficiency anemia (IDA) and evaluating iron response in infants with moderate acute malnutrition (MAM).** This study concluded that this parameter was not influenced by MAM or inflammation; and it alone can be used to detect IDA and monitor treatment response in infants with MAM.(14)

Clin Chem. Study is **Iron deficiency and erythropoiesis: new diagnostic approaches**. This article highlights the use of red cell and reticulocyte cellular indices, which reflect in almost real time the development of iron deficiency and the response to iron therapy.(15)

J Lab Physicians. Study is about **Automated hematology analyzers**: Recent trends and applications. They provide the new information about new parameters in automated analyzers like Beckman-Coulter DxH800.(16)

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III. METHODOLOGY &METHODS

METHODOLOGY :

Site of the study : Central laboratory of Sri Ramachandra higher education and Research.

Type of study : Retrospective

Period of the study : Three (3) months

Sample size : 100 normal individuals,100 patients with iron deficiency anemia.

Inclusion criteria : Samples with Serum iron levels below <30 µg/dL and or serum ferritin levels below <15 µg/L.

Exclusion criteria : Samples with inadequteinformation ,Subjects who had histories of blood malignancies, renal disease, Immune thrombocytopenia and thalassemia were excluded from this study.

METHOD

IRON PROFILE:

Serum Iron :

Blood collected in red tube(without anticoagulant) test is performing in Beckman coulter Au 5800 by using the method 2,4,6-Tripyridyl-S-triazine (**TPTZ**) is the most widely used analytical reagent for the spectrophotometric determination of iron.

Normal Range : Male ranges of around 65 to 176 µg/dL

Female ranges of 50 to 170 µg/dL

Serum Ferritin :

Blood collected in red tube(without anticoagulant) test is performing in Beckman coulter Au 5800 by using the method Chemiluminescence Immunoassay (**CLIA**)is the most widely used analytical method for the spectrophotometric determination of ferritin.

Normal Range : 20 to 250ng/mL for males

10 to 120 ng/mL for females.

Transferrin (saturation levels) :

Blood collected in red tube(without anticoagulant) test is performing in Atalica by using the method Nephelometry principles.

Calculation is done by the formula (Serum Transferrin(saturation)= Iron /TIBC*100) Normal Range :204–360 mg/dL

Total Iron Binding Capacity :

Blood collected in red tube(without anticoagulant) test is performing in Beckman coulter Au 5800 by using the spectrophotometric method determination of Total Iron Binding Capacity (TIBC).

Calculation is done by the formula (Serum TIBC = UIBC+Iron) Normal Range :262–474 mcg/dL

COMPLETE BLOOD COUNT :

Blood collected in EDTA test is performing in UnicelDxH 800 (Beckman coulter) by using the method impedance for red cell count, spectrophotometric method using Sodium lauryl Sulphate for Hemoglobin estimation MCV derived from Histogram, other red cells and extended RBC parameters are calculated.

EXTENDED RED CELLA PARAMETERS :

Microcytic anemia factor

New calculate parameter-Maf(Microcyticanemia factor) have been obtained by UnicelDxH 800 (Beckman coulter) . Diagnostic use-fullness of Mafhas been tested in early recognition of iron deficient in some specific pathological conditions such as congenital heart disease and chronic kidney disease.

Calculation = (Maf= ((Hb*MCV)/100))

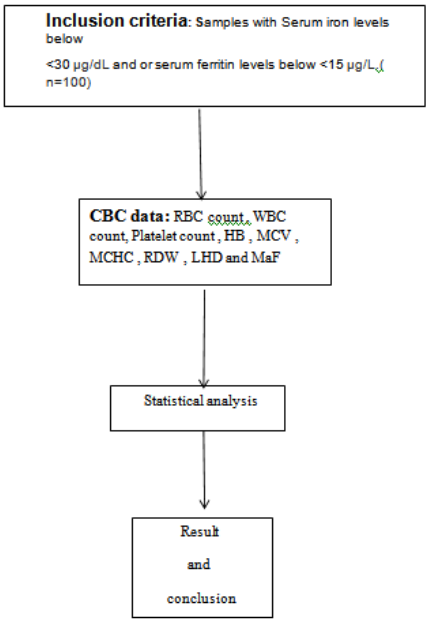
Low Density Hemoglobin

New calculate parameter-Low haemoglobin density (LHD%) have been obtained by UnicelDxH 800 (Beckman coulter) . Diagnostic use-fullness of LHD%has been tested in early recognition of iron deficient in some specific pathological conditions such as congenital heart disease and chronic kidney disease.

Calculation = LHD%=[100√ 1 1= 1 e1:8(30-MCHC) ½]

PLAN ANALYSIS

Plan analysis



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STASTICAL ANALYSIS

GNDER

Male	Female
46	54

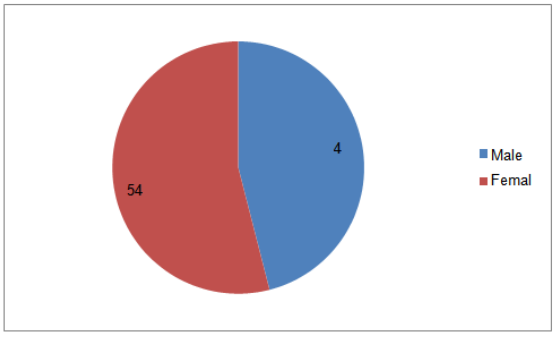

Figure :1 To find IDA among the 100 patients data ,where males are 46 and females are 54 .They showed in the diagram.

Age Distribution

Total number of patients is 100,where men is 46 and women is 54.

AGE	MALE	FEMALE
0-20	2	1
20-40	7	22
40-60	18	18
60-80	15	12
80-100	4	1

Figure :2 Finding the age between the male and female by the age intervals from 0-100 in between 20 .

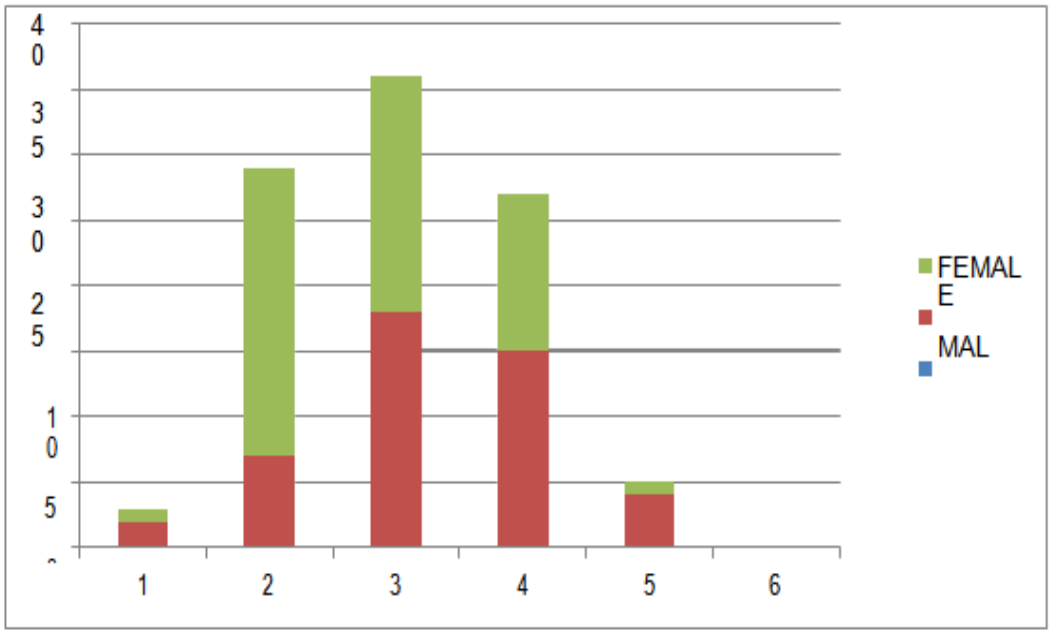

Figure: 3 Age Distribution bar chart shown light green colour indicates the female ,brown colour indicate males and blue colour shows the age intervals.

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T-Test

Group Statistics

Gp		N	Mean	Std. Deviation	Std. Error Mean
MAF1	abnormal	50	6.2047	2.01848	.28546
	normal	50	7.6575	2.20319	.31158
LHD	abnormal	50	28.3805	30.99765	4.38373
	normal	50	11.0601	16.92066	2.39294

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
MAF1	Equal variances assumed	.001	.981	-3.438	98	.001	-1.45286	.42257	-2.29144	-.61428
	Equal variances not assumed			-3.438	97.258	.001	-1.45286	.42257	-2.29152	-.61420
LHD	Equal variances assumed	27.096	.000	3.468	98	.001	17.32045	4.99432	7.40938	27.23152
	Equal variances not assumed			3.468	75.820	.001	17.32045	4.99432	7.37301	27.26789

ROC Curve

Case Processing Summary

Gp	Valid N (listwise)
Positive[a]	50
Negative	50

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Smaller values of the test result variable(s) indicate stronger evidence for a positive actual state.

a. The positive actual state is abnormal.

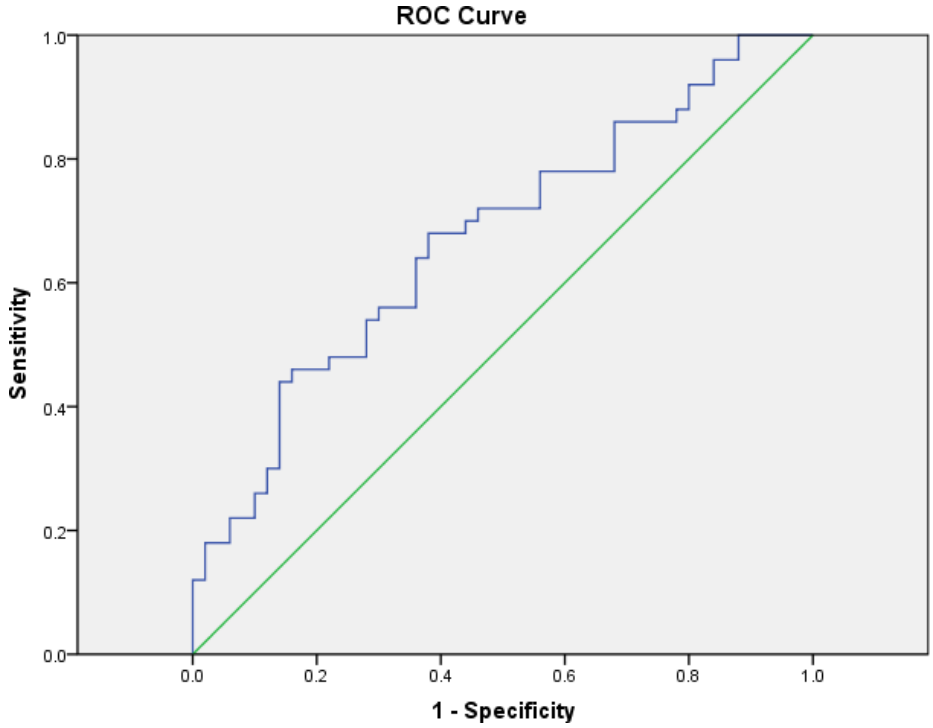

Figure :4 ROC curves of shortlisted red cell parameters to find the IDA. Maf above the cut off point smallest values is minus 1 and largest values is plus 1 (AUC: 0.671; 95% CI: 0.566- 0.776; Sensitivity: 86%; Specificity: 88%)

Area Under the Curve

Test Result Variable(s):MAF1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.671	.054	.003	.566	.776

a.Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

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Coordinates of the Curve

Test Result Variable(s):MAF1

Positive if Less Than or Equal To[a]	Sensitivity	1 – Specificity
.5660	.000	.000
1.7334	.020	.000
1.9502	.040	.000
2.6873	.060	.000
3.4015	.080	.000
3.4493	.100	.000
3.6673	.120	.000
4.1064	.120	.020
4.3648	.140	.020
4.4080	.160	.020
4.5021	.180	.020
4.5711	.180	.040
4.6225	.180	.060
4.7092	.200	.060
4.7889	.220	.060
4.8600	.220	.080
4.9558	.220	.100
5.0389	.240	.100
5.0595	.260	.100
5.1119	.260	.120
5.1741	.280	.120
5.2079	.300	.120
5.2513	.300	.140
5.2978	.320	.140
5.3307	.340	.140
5.3765	.360	.140
5.4357	.380	.140
5.4597	.400	.140
5.6315	.420	.140
5.8616	.440	.140
5.9350	.440	.160
5.9503	.460	.160
5.9638	.460	.180

5.9899	.460	.200
6.0133	.460	.220
6.0542	.480	.220
6.1311	.480	.240
6.1893	.480	.260
6.2245	.480	.280
6.2523	.500	.280
6.2817	.520	.280
6.3173	.540	.280
6.3284	.540	.300
6.3944	.560	.300
6.4931	.560	.320
6.5499	.560	.340
6.6129	.560	.360
6.6648	.580	.360
6.7279	.600	.360
6.8379	.620	.360
6.9195	.640	.360
7.0256	.640	.380
7.1267	.660	.380
7.1527	.680	.380
7.1797	.680	.400
7.2111	.680	.420
7.2368	.680	.440
7.2628	.700	.440
7.3002	.700	.460
7.3313	.720	.460
7.3571	.720	.480
7.3978	.720	.500
7.4266	.720	.520
7.4877	.720	.540
7.5587	.720	.560
7.5847	.740	.560
7.6809	.760	.560
7.8179	.780	.560
7.8696	.780	.580
7.8837	.780	.600
7.9659	.780	.620
8.0430	.780	.640
8.0676	.780	.660

8.0940	.780	.680
8.1191	.800	.680
8.2027	.820	.680
8.2706	.840	.680
8.2756	.860	.680
8.2820	.860	.700
8.2935	.860	.720
8.3371	.860	.740
8.3968	.860	.760
8.4342	.860	.780
8.6080	.880	.780
8.7987	.880	.800
8.8395	.900	.800
8.8883	.920	.800
8.9791	.920	.820
9.0631	.920	.840
9.1310	.940	.840
9.2105	.960	.840
9.6846	.960	.860
10.1492	.960	.880
10.1824	.980	.880
10.1954	1.000	.880
10.8863	1.000	.900
11.6209	1.000	.920
11.9750	1.000	.940
12.5942	1.000	.960
13.7309	1.000	.980
15.5468	1.000	1.000

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

ROC Curve

Case Processing Summary

gp	Valid N (listwise)
Positive[a]	50

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Negative	50

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.

a. The positive actual state is abnormal.

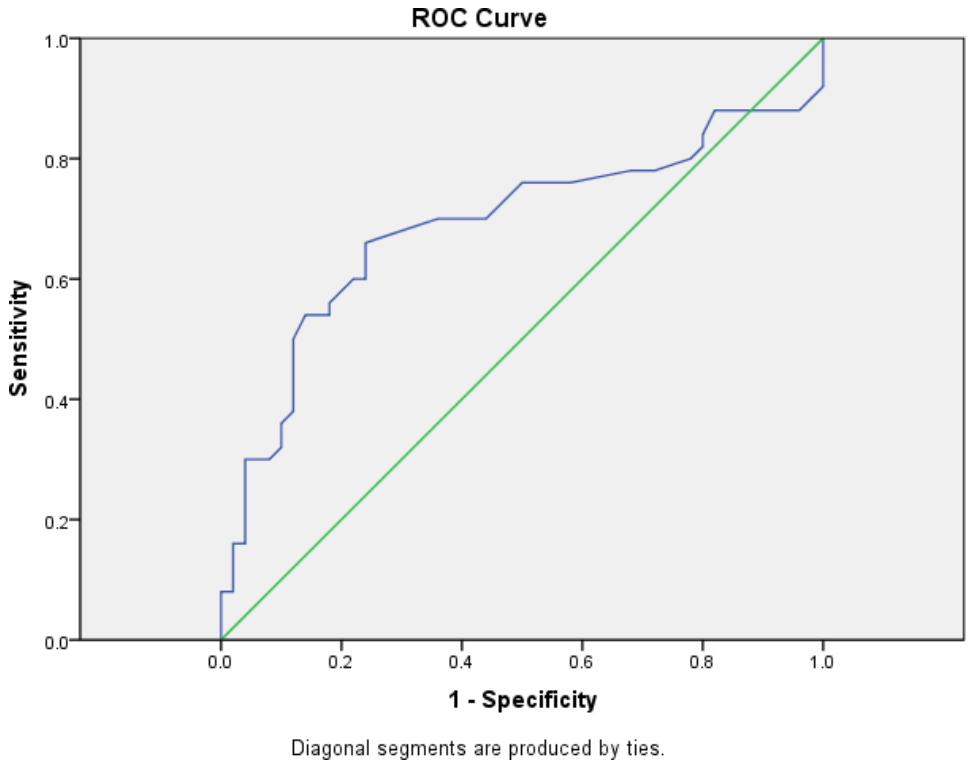

Figure :5 ROC curves of shortlisted red cell parameters to find the IDA. LHD% above the cut off point is tie between positive and negative groups (AUC: 0.688; 95% CI: 0.579-0.797; Sensitivity:89%; Specificity: 97%).

Area Under the Curve

Test Result Variable(s):LHD

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.688	.056	.001	.579	.797

The test result variable(s): LHD has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

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Coordinates of the Curve

Test Result Variable(s):LHD

Positive if Greater Than or Equal To[a]	Sensitivity	1 – Specificity
-.0721	1.000	1.000
.9715	.980	1.000
1.0630	.960	1.000
1.1631	.940	1.000
1.2726	.920	1.000
1.5359	.900	.980
1.9137	.880	.960
2.2909	.880	.920
2.6139	.880	.880
2.8599	.880	.820
3.1289	.840	.800
3.4233	.820	.800
3.7452	.800	.780
4.0973	.780	.720
4.4825	.780	.700
4.9036	.780	.680
5.3641	.760	.580
5.8676	.760	.500
6.4180	.700	.440
7.0195	.700	.360
7.6768	.660	.240
8.3949	.600	.240
9.1791	.600	.220
10.0351	.560	.180
10.9692	.540	.180
11.9879	.540	.140
13.0982	.500	.120
14.3074	.460	.120
15.6231	.440	.120
17.8610	.420	.120
20.2882	.400	.120
23.1288	.380	.120
27.3682	.360	.100
32.2364	.340	.100
36.2470	.320	.100

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39.1543	.300	.080
42.2056	.300	.040
45.3869	.280	.040
48.6786	.220	.040
52.0564	.200	.040
55.4908	.180	.040
63.9645	.160	.040
72.2633	.160	.020
75.2824	.140	.020
78.1178	.120	.020
84.7004	.080	.020
94.1588	.080	.000
98.5323	.060	.000
98.7696	.040	.000
99.4390	.020	.000
100.9999	.000	.000

The test result variable(s): LHD has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

IV. RESULT

A total of 50 normal subjects and 50 subjects diagnosed with iron deficient disorders were considered for this study.

Values are taken based on inclusion criteria and exclusion criteria.

In accordance with the T – test ,two set of group LHD% and MaF were analysed and the mean values of LHD% is higher than the MaF.

ROC curves were generated for only shortlisted eRBC parameters which harbored statistical differences ($P<0.05$) and were deemed clinically significant.

Within this result section .Subsequently only ROC curves which yielded AUC >0.8 were displayed to emphasize on high diagnostic power of the parameters display the ROC curves constructed to visualizes the AUD of each of the tested parameters to find IDA.

Cut-off points were also determined while maintaining good sensitivity and specificity values.

Mean of MaF in normal values is 7.6575 and for abnormal values is 6.2047. Mean of LHD% in normal values is 11.0601 and for abnormal values is 28.3805. According to Levene's test for equality of variances for

Maf = sig .981 LHD% = sig .000

ROC curve analysis for MAF (showed in the figure 4) which is a Unicel DxH800 parameter yielded the best AUC to find IDA from healthy individuals, above the cut off point smallest values is minus 1 and largest values is plus 1 (AUC: 0.671; 95% CI: 0.566-0.776; Sensitivity: 86%; Specificity: 88%) and LHD% also Unicel DxH 800 parameter it also yielded the best AUC (showed in the figure 5) cut off point is tie between positive and negative groups (AUC: 0.688; 95% CI: 0.579-0.797; Sensitivity:89%; Specificity: 97%).

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V. DISCUSSION

Uncomplicated IDA can usually be recognized by correlating the red cell indices and biochemical markers of negative iron balance ,including reduced Serum Ferritin , Serum Iron and Transferrin saturation.

Functional iron deficiency is defined as an imbalance between the iron needed for erythropoiesis and the iron supply with the latter not maintained at sufficient rate for adequate hemoglobinization of reticulocytes and erythrocytes. Efforts have been made to evaluate some readily available and relatively inexpensive laboratory parameter as indirect markers of iron-restricted erythropoiesis and iron availability in a clinical context influenced by inflammation and acute phase reaction.

The assessment of iron requirements and monitoring of therapy require accurate markers. The best combination of haematological indices for iron deficiency is an increased % of hypochromic erythrocytes and a reduced HB content of reticulocytes.

In a similar study conducted by Angeli Ambayye MAF was shown to utilizing extendedRBC parameters to distinguishing Iron deficient erythropoiesis from healthy subjects which support our findings, According to their study based on MAF,which is a Unicel DxH 800 parameter yielded the best AUC to distinguish LID from IDA above the cut-off point of 9.25 (AUC: 0.954; 95% CI: 0.927-0.980; Sensitivity:89.02%; Specificity: 88.46%). (1)

In a similar study conducted by Dopsaj, et al. MAF was shown to effectively distinguish iron- deficient athletes from healthy subjects, which support our findings. As a Sysmex XE-5000 parameter, %MicroR also generated the best AUC to distinguish IDA from LID above the
cut-off point of 0.655% (AUC: 0.915; 95% CI: 0.874-0.956; Sensitivity: 85.57%; Specificity: 86.58%).(2)

According to E. H. Y. NG, J. H. W. LEUNG, Y. S. LAU, E. S. K. MA Beckman Coulter DxH800 in distinguishing iron deficiency anaemia from thalassaemia trait

Statistically, MAF was the best parameter of their study. With a cut-off of 6.8, the area under the curve (AUC) was 0.817, the sensitivity was 55.4%, and the specificity was 93%.(3)

ROC curve analysis of **Dopsaj** study for Maf_ in the diagnosis of iron-deficient erythropoiesis in elite athletes indicates sensitivity of 61.5%, and specificity of 93.0%, with AUC = 0.826 (CI 95% 0.754–0.885, P < 0.001) and on cut off value 114.(4)

ROC analysis of LHD% and Maf based on **Ashutosh Singh, Rajendra Chaudhary, Hem C Pandey, Atul Sonker** their study 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 51% and 81.7%, AUC was 0.646 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 with a sensitivity and specificity of 54.9% and 93%, AUC was 0.646 , respectively.(5)

Several parameters yielded excellent AUC, such as MAF to distinguish LID from IDA approximately (AUC: 0.954; 95% CI: 0.927-0.980; Cut-off: > 9.25; Sensitivity: 89.02%; Specificity: 88.46%) and %MicroR to distinguish IDA from LID (AUC: 0.915; 95% CI: 0.874-0.956; Cut-off: > 0.655%; Sensitivity: 85.57%; Specificity: 86.58%).

The utility of eRBC parameters is not only limited to distinguishing iron-deficiency related disorders, as shown in a similar study by Torino, et al. which reported %Hypo-He and
%MicroR as a parameter of interest in distinguishing anemia of chronic disease (ACD) from IDA Other research groups have also considered the utilization of various red cell parameters in characterizing hematological diseases, such as nonsickling hemoglobinopathy], chronic nephropathies and even mortality in subjects undergoing hemodialysis.

In future ,simple algorithms may even be formulated by arithmetically incorporating more than one routine (or) eRBC parameters to archieve better distinction between 2 conditions of interest with the aid of ROC curves.

VI. CONCLUSION

The advent of newer generation of CBC analyzers has unveiled possibilities to utilize such technology in providing a surrogate first line indication of iron deficiency related disorders ,especially in facilities which lack resources (or) access of gold standard diagnostic method.

Beside parameters that describe iron metabolism dynamics (body iron and soluble transferring receptor LHD % indicator of hypochromic have the highest potential to differentiated and classify in patients with congenital heart disease.

From data of the ROC analysis, MAf seems of limited value for iron depletion detection,

but performs well in detecting iron-deficient erythropoiesis, adding value with no additional cost.

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APPENDIX

PROFORMA

Unique ID :
Hospital ID :
Age :
Sex :

Provisional diagnosis :**COMPLETEBLOOD COUNT**
RBC count**:**
WBC count**:**
Platelet count**:**
HGB**:**
MCV**:**
MCHC**:**
RDW**:**

EXTENDED RBC(eRBC)PARAMETERS
Low Hemoglobin Density (LHD)**:** Microcytic Anemia Factor (MAF)**:**
IRON PROFILE

A. Serum Iron**:**

B. Serum Ferritin**:** C.Transferrin (Saturation)**:** D.Total Iron Binding Capacity**:**