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## **Original Research Article**

# Novel reticulocyte parameters in thalassemia and iron deficiency anemia

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#### ABSTRACT

**Introduction:** Reticulocytes are immature, non-nucleated RBCs that still contain RNA. It provides useful information about the capacity of the bone marrow to synthesize and release red cells in response to anemia and helps to distinguish between decreased RBC production and enhanced peripheral destruction.

**Materials and Methods:** A prospective study was done between thalassemia and iron deficiency anemia cases. The duration of the study was 1 year where 139 cases were included. In our study, a total of 139 patients are included out of which 94 cases were of Thalassemia and 45 cases of Iron deficiency anemia. Measurement of complete blood count (CBC) with reticulocyte fractions was done in the SYSMEX XN-550 system and for serum ferritin, Architect iSystem was used.

**Results:** This study demonstrates the cut-off value of IRF for detecting Thalassemia trait as 15.85-21.50%, IDA was >21.50% and for Thalassemia major it was < 15.85%. The LFR% optimum cut-off for detecting Thalassemia trait was estimated to be 83.1-84.2%, IDA <83.1% and for Thalassemia major it was > 84.2%. The MFR% optimum cut-off for detecting Thalassemia trait was estimated to be <12.9%, IDA was >13.25% and for Thalassemia major it was 12.9-13.25%. The HFR% optimum cut-off for detecting Thalassemia trait was estimated to be 2.4-5.3%, IDA was >5.3% and for Thalassemia major it was < 2.4%. **Conclusion:** The use of new hematological parameters and doing further comparative-based studies will help in differentiating various types of anemia and give knowledge of physiopathology of the underlying disease

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## 1. Introduction

Reticulocytes are immature, non-nucleated red blood cells (RBCs) that still contain ribonucleic acid (RNA). It provides useful information about the capacity of the bone marrow to synthesize and release red cells in response to anemia and helps to distinguish between decreased RBC production and enhanced peripheral destruction.

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Iron deficiency anemia (IDA) and thalassemia trait (TT) are the most common cause of microcytosis and their clinical management is quite different; so, the discrimination between thalassemic and non-thalassemic microcytosis has important implications. <sup>1,2</sup>

Microcytosis accompanied by a high RBC count, normal red cell distribution width (RDW) and an elevated level of HbA2 are suggestive of Beta Thalassemia Trait (BTT). <sup>3–5</sup> Microcytosis accompanied by a low ferritin value suggests iron deficiency. <sup>6,7</sup>

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Both IDA & TT show microcytosis and in pregnancy they are indistinguishable. So this study aims to study the newer reticulocyte parameter and their role in differentiating between iron deficiency anemia and thalassemia trait.<sup>6</sup>

Till recent times, reticulocyte count was done manually however current hematology analyzer uses fluorescent dyes that bind to RNA allow for many more cells to be analyzed, thereby increasing the accuracy and precision of reticulocytes count. Most hematology analyzers offer automated reticulocyte counting and can report reticulocyte numbers with routine CBC parameters. 8 Coefficient of variation (CV) of 10% or less can be achieved by using automated analyzers.

Current instruments also have the capability to report novel reticulocyte parameters such as immature reticulocyte fraction (IRF) and reticulocyte cellular indices such as cell volume and Hb content. <sup>9</sup> However, the clinical utility of these novel parameters is still being investigated.

Besides measurement of the reticulocyte hemoglobin (RET-He) content is helpful in detecting early stages of iron deficiency prior to the development of anemia. 1 Effective erythropoiesis can be monitored by quantitative measurement of reticulocytes. 10,11 The amount of RNA in these cells can be assessed with flow cytometry and divided into low- (LFR), middle- (MFR) and highfluorescence reticulocytes (HFR). This distribution is correlated with their maturation. HFR fraction represents the most immature reticulocytes. Immature reticulocyte fraction (IRF) includes HFR and MFR. Currently it is used as a predictor of effective erythropoiesis in autologous bone marrow transplantation and for granulocyte recovery after polychemotherapy in leukaemic patients. Few studies have suggested that HFR is the strongest predictor of hematological recovery alone but others also suggested IRF as a strong predictor of hematological recovery. 12

RET- He is the cellular hemoglobin content of reticulocytes. It correlates with iron deficient erythropoiesis and is a very useful marker of iron deficiency in infants, children, adult blood donors, old age group, pregnant women and Chronic Kidney disease (CKD) patients undergoing hemodialysis. RET-He is a useful indicator for monitoring patient's response to iron therapy and detecting iron-restricted erythropoiesis in patients receiving erythropoietin therapy. It also reflects the recent functional availability of iron for erythropoiesis.

#### 2. Materials and Methods

A prospective study was done between Thalassemia and iron deficiency cases. The duration of the study was 1 year where 139 cases were included. The study was done at King George's Medical University, Lucknow, and Uttar Pradesh, India. The study is approved by the Ethical committee (King George's Medical University U.P., Institutional Ethics Committee) with approval number (1755/Ethics/19).

Consent for the participation in this study was taken from patient/ relative in hindi or english. The inclusion criteria is patients with Iron deficiency anemia (IDA) and Thalassemia, of all age groups. The exclusion criteria includes cause of anemia other than IDA and Thalassemia and patients who refused for consent. Blood samples were collected in EDTA vial (Ethylenediamine tetra-acetic acid) and plain vial. For measurement of complete blood count (CBC) with reticulocyte fractions were done in the SYSMEX XN-550 system and for serum ferritin, Architect iSystem was used.

#### 3. Results

The cases were divided into 4 groups and intergroup comparison was done for various parameters as mentioned in Table 1.

Hb, Hemoglobin; IDA, Iron deficiency anemia; RBC, Red blood cells; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; RDW-CV, Red cell distribution width - coefficient of variation; RET%, Reticulocyte count; IRF, Immature reticulocyte fraction; LFR, Low fluorescence ratio; MFR, Medium fluorescence ratio; HFR, High fluorescence ratio; RET-He, Reticulocyte hemoglobin equivalent.

The mean Hb level in Mild IDA and Thalassemia trait groups was higher in comparison to severe IDA and Thalassemia major groups. A significant difference was found in the mean Hb level among the four groups (p<0.001). The mean RBC level in Mild IDA and Thalassemia trait groups was higher in comparison to severe IDA and Thalassemia major groups. A significant difference was found in the mean RBC level among the four groups (p<0.001). The mean Mean corpuscular volume (MCV) level was highest in Thalassemia major group and lowest in the Thalassemia trait group. A significant difference was found in the mean MCV (fL) level among the four groups (p<0.001). The mean Mean corpuscular hemoglobin (MCH) level was highest in Thalassemia major group and lowest in the Thalassemia trait group. A significant difference was found in the mean MCH level among the four groups (p<0.001). The mean Mentzer index was highest in Thalassemia major group and lowest in the Thalassemia trait group. A significant difference was found in the mean Mentzer index among the four groups (p<0.001). The mean RDW-CV (%) level was highest in Thalassemia major group and lowest in the Thalassemia trait group. A significant difference was found in the mean RDW (%) level among the four groups (p<0.001). The mean Reticulocyte count (RET%) level was highest in Thalassemia major group and lowest in the mild IDA group. A significant difference was found in the mean RET% level among the four groups (p<0.001). The mean IRF level was highest in the Severe IDA group and lowest in Thalassemia major group. A significant difference was found in the mean IRF level

Table 1: Intergroup comparison of various parameters

Parameter	<b>Group</b> Mild IDA	<b>Mean</b> 10.20	<b>SD</b> 0.22	p-value	
НЬ	Severe IDA	7.53	1.46	0.001	
	Thalassemia trait	11.74	1.70	< 0.001	
	Thalassemia major	8.20	2.01		
RBC (106/uL)	Mild IDA	3.23	0.49		
	Severe IDA	2.98	0.72	-0.001	
	Thalasemia trait	5.68	0.94	< 0.001	
	Thalasemia major	2.49	0.59		
MCV (fL)	Mild IDA	76.30	2.34		
	Severe IDA	78.99	15.53	<b>~</b> 0.001	
	Thalassemia trait	64.39	6.88	< 0.001	
	Thalassemia major	85.54	16.68		
	Mild IDA	24.95	1.35		
ACII ( )	Severe IDA	26.19	10.08	-0.001	
MCH (pg)	Thalassemia trait	20.48	2.26	< 0.001	
	Thalassemia major	31.86	3.77		
	Mild IDA	24.06	3.92		
A	Severe IDA	27.62	9.52	-0.001	
Mentzer index	Thalassemia trait	11.73	3.51	< 0.001	
	Thalassemia major	36.34	11.35		
	Mild IDA	21.20	4.00		
	Severe IDA	19.01	2.06	<0.001	
RDW-CV (%)	Thalassemia trait	18.12	2.19		
	Thalassemia major	21.35	4.18		
	Mild IDA	0.68	0.35		
NETT CT	Severe IDA	0.91	0.40	0.001	
RET%	Thalassemia trait	1.65	0.51	< 0.001	
	Thalassemia major	4.57	3.59		
	Mild IDA	23.38	3.89		
D.E.	Severe IDA	24.77	14.02	0.001	
RF	Thalassemia trait	16.46	8.50	< 0.001	
	Thalassemia major	15.09	8.76		
	Mild IDA	74.85	4.33		
	Severe IDA	75.56	12.74		
LFR%	Thalassemia trait	83.43	8.49	< 0.001	
	Thalassemia major	85.19	8.91		
	Mild IDA	15.68	4.48		
HTD cr	Severe IDA	16.42	9.16	2 225	
MFR%	Thalassemia trait	12.37	5.00	0.009	
	Thalassemia major	11.51	6.86		
	Mild IDA	7.70	2.14		
IED &	Severe IDA	8.35	6.73	2.22	
HFR%	Thalasemia trait	4.09	3.89	< 0.001	
	Thalasemia major	3.58	3.65		
RET-HE (pg)	Mild IDA	20.05	3.31		
	Severe IDA	20.39	3.78		
	Thalassemia trait	19.45	3.14	< 0.001	
	Thalassemia major	16.88	3.27		
	Mild IDA	6.41	6.77		
	Severe IDA	14.62	7.75		
S.Ferrittin	Thalassemia trait	150.39	367.89	< 0.001	
	Thalassemia major	1806.32	442.91		

among the four groups (p<0.001). The mean LFR% level was highest in Thalassemia major group and lowest in the mild IDA group. The significant difference was found in mean LFR% level among the four group (p<0.001). The mean MFR% level was highest in Severe IDA group and lowest in Thalassemia major group. The significant difference was found in mean MFR% level among the four group (p=0.009). The mean HFR% level was highest in Severe IDA group and lowest in Thalassemia major group. The significant difference was found in mean HFR% level among the four group (p<0.001). The mean RET-HE level was highest in Severe IDA group and lowest in Thalassemia major group. The significant difference was found in mean RET-HE level among the four group (p<0.001). The mean S.Ferritin level was highest in Thalassemia major group and lowest in Mild IDA group. The significant difference was found in mean S.Ferritin level among the four group (p<0.001).

#### 4. Discussion

This comparative observational study was conducted at the Department of Pathology in collaboration with the Department of Clinical Haematology and Department of Pediatrics, King George's Medical University, Lucknow, Uttar Pradesh. The study aims to assess the automated reticulocyte parameters in Iron Deficiency Anemia and Thalassemia. In our study, a total of 139 patients have included out of which 94 cases were of Thalassemia and 45 cases of Iron deficiency anemia.

In our study, we divided the cases into 4 groups, Mild IDA, Severe IDA, Thalassemia Trait, and Thalassemia Major. IDA patients with Hb levels <10g/dl were put under severe IDA and Hb levels ranging from 10-12 g/dl were put under Mild IDA. C. Ceylan et al. 9 and Urrechaga et al. 13 also divided their IDA group into mild IDA and severe IDA with Hb <10 g/dl and 10-12 g/dl.

We compared our study with Urrechaga et al. <sup>13</sup> hematological and biochemical findings and found similar findings in mean RBC count, mean Hb level, mean MCV, mean MCH, mean RDW-CV, and mean RET-He (Table 2).

The above Table 3 shows similar values observed by C.Ceylan et al. 9 for mean Hb and mean Ret He.

The mean Mentzer index was highest in Thalassemia major group and lowest in the Thalassemia trait group. A significant difference was found in the mean Mentzer index among the four groups (p<0.001). The mean Mentzer index in the case of thalassemia trait is 11.73±3.51 and the other group had a mean index >13. Bi group comparison for the Mentzer index showed significant differences in mild IDA vs Thalassemia trait (p=0.012) and severe IDA vs Thalassemia trait (p<0.001). All bigroups showed significant differences among all the pairs Except Mild IDA vs Severe IDA where the difference was not found to be significant (p=0.812). With Hb level, RET%

showed a significant positive correlation (r=0.470, p=0.012) in Thalassemia major group. IRF showed significant negative correlation (r=-0.197, p=0.020) overall, HFR% too showed significant negative correlation overall (r=-0.201, p=0.018) and RET-HE showed significant positive correlation (r=0.311, p=0.011) in Thalassemia trait group.

On ROC analysis, IRF optimum cut-off for detecting Thalassemia trait was estimated to be 15.85-21.50%, IDA was >21.50% and for Thalassemia major it was < 15.85%. Sensitivity and specificity for detecting Thalassemia trait were 75.5% & 66.7% respectively and for Thalassemia major it was 60% & 47% respectively. The LFR% optimum cut-off for detecting Thalassemia trait was estimated to be 83.1-84.2%, IDA <83.1% and for Thalassemia major it was > 84.2%. Sensitivity and specificity for detecting Thalassemia trait were 60.6% & 80.0% respectively and for Thalassemia major it was 60.6% & 80% respectively. The MFR% optimum cut-off for detecting Thalassemia trait was estimated to be <12.9%, IDA was >13.25% and for Thalassemia major it was 12.9-13.25%. Sensitivity and specificity for detecting Thalassemia trait were 59.6% & 77.8% respectively and for Thalassemia major it was 75% & 45.5% respectively. The HFR% optimum cut-off for detecting Thalassemia trait was estimated to be 2.4-5.3%, IDA was >5.3% and for Thalassemia major it was < 2.4%. Sensitivity and specificity for detecting Thalassemia trait were 75.5% & 66.7% respectively and for Thalassemia major it was 71.4% & 53% respectively.

The drawback of this study is the limited available cases of Thalassemia and Iron deficiency anemia. Only Beta thalassemia trait and Beta-thalassemia major cases were included among thalassemia.

#### 5. Conclusions

We conclude with the study of automated reticulocyte parameters along with other hematological and biochemical indices in Iron deficiency anemia and Thalassemia. Its usefulness and limitations in differentiating between Iron deficiency anemia, Thalassemia trait, and Thalassemia major. This study demonstrates the cut-off value of IRF detecting Thalassemia trait was estimated to be 15.85-21.50%, IDA was >21.50% and for Thalassemia major it was < 15.85%. The LFR% optimum cutoff for detecting Thalassemia trait was estimated to be 83.1-84.2%, IDA <83.1% and for Thalassemia major it was >84.2%. The MFR% optimum cut-off for detecting Thalassemia trait was estimated to be <12.9%, IDA was >13.25% and for Thalassemia major it was 12.9-13.25%. The HFR% optimum cut-off for detecting Thalassemia trait was estimated to be 2.4-5.3%, IDA was >5.3% and for Thalassemia major it was < 2.4%.

The use of new hematological parameters and doing further comparative-based studies will help in differentiating various types of anemia and give knowledge

Table 2: Comparative study of Hematological and Biochemical data

	Thalassemia Trait mean (SD)		Mild IDA mean(SD)		Severe IDA mean (SD)	
	Urrechaga et al. <sup>13</sup>	our study	Urrechaga et al. <sup>13</sup>	our study	Urrechaga et al. <sup>13</sup>	our study
$RBC(10^{12}/L)$	5.80(0.53)	5.68(0.94)	4.81(0.47)	3.23(0.49)	4.17(0.58)	2.98(0.72)
Hb(g/dl)	11.9(11.3)	11.74(1.70)	11.0(7.5)	10.20(0.22)	9.0(9.1)	7.53(1.46)
MCV(fL)	64.6(3.4)	64.39(6.88)	75.3(4.8)	76.30(2.34)	73.2(6.7)	78.99(15.53)
MCH(pg)	20.7(1.1)	20.48(2.26)	21.5(1.8)	24.95(1.35)	21.7(2.7)	26.19(10.08)
RDW-CV%	15.7(1.0)	18.12(2.19)	17.7(2.5)	21.20(4)	17.7(2.3)	19.01(2.06)
RET%	13.4(4.7)	1.65(0.51)	11.1(4.7)	0.68(0.35)	13.6(8)	0.91(0.40)
IRF%	8.7(5)	16.46(8.50)	12.9(5.8)	23.38(3.89)	16.7(6.2)	24.77(14.02)
RET He(pg)	22.1(1.6)	19.45(3.14)	25.3(2.9)	20.05(3.31)	22.3(3.7)	20.39(3.78)
Ferritin(ug/L)	116(97)	150.39(367.89)	17(22)	6.41(6.77)	13(15)	14.62(7.75)

RBC, Red blood cells; Hb, Hemoglobin; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; RDW-CV%, Red cell distribution width - coefficient of variation; IRF; Immature reticulocyte fraction; RET-He, Reticulocyte hemoglobin equivalent; Urrechaga et al. <sup>13</sup>

**Table 3:** Comparative study of Hb and Ret He values with C.Ceylan et al. <sup>9</sup>

	Thalassemia T	Thalassemia Trait mean(SD)		Mild IDA mean(SD)		Severe IDA mean(SD)	
	C.Ceylan et al. 9	our study	C.Ceylan et al. 9	our study	C.Ceylan et al. <sup>9</sup>	our study	
Hb(g/dl)	11.1	11.74	11.4	10.2	8.7	7.53	
Ret He(pg)	21	19.45	24	20.05	19.2	20.39	

Hb, Hemoglobin; Ret He, Reticulocyte hemoglobin equivalent; C.Ceylan et al. 9

of physiopathology of the underlying disease. It will also help the physician provide good patient care with routine laboratory tests reported by automated analyzers. With the advancement of automation and computerization, hematology has become more advance in diagnosing diseases and helping clinicians in giving precise clinical diagnosis and going for different treatment spectrums to a disease.

#### 6. Conflict of Interest

None.

## 7. Source of Funding

None.

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