

# Efficacy of Reticulocyte Hemoglobin Content and Immature Reticulocyte Fraction in Early Diagnosis of Latent Iron Deficiency in Blood Donors

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## ABSTRACT

**Objective:** To assess the utilization of Reticulocyte Hemoglobin content and Immature Reticulocyte Fraction in detecting of Latent Iron deficiency in Blood donors.

**Methodology:** A prospective Cross-sectional study was conducted at Pakistan Atomic Energy Commission Hospital in collaboration with Pakistan Institute of Medical sciences, Islamabad Pakistan. The study was conducted over a period of 6 months from July 2023 to December 2023. A total of 191 donors were included in study and evaluated by using Mindray BC-6200 hematology analyzer. Serum Ferritin was correlated with Reticulocyte parameters. The agreement between Ferritin and Mindray BC-6200 was assessed by applying P- test.

**Results:** The mean age of donor group was 36.35 years with predominantly male donors (82%). About 65% were repeated donors. The sensitivity of RHE in correlation with serum ferritin was 87.5% and Specificity of this test was 93.7% and the sensitivity of IRF in correlation with serum ferritin was 68.7% and Specificity of this test was 86.75%. The P-value is significant in both parameters <0.01.

**Conclusion:** RHE can be used as a first line test in detecting Latent Iron deficiency in donors and can replace serum Ferritin, IRF is less sensitive and specific as compared to RHE.

**Key words:** blood donors, latent iron deficiency, reticulocyte haemoglobin expression (RHE), immature reticulocyte fraction (IRF), serum ferritin

### Authors' Contribution:

<sup>1,2</sup>Conception; Literature research; manuscript design and drafting; <sup>3,4</sup>Critical analysis and manuscript review; <sup>5,6</sup>Data analysis; Manuscript Editing.

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## Introduction

According to World Health Organization (WHO), anemia can be defined as a condition in which the amount of red blood cells or their oxygen-carrying capacity is deficient to meet the functional needs of the body<sup>1,3</sup>. Worldwide the commonest cause of anemia is iron deficiency. Donation of a single unit of whole blood results in a loss of 200–250 mg iron from the donor's body iron stores<sup>3,4,5</sup>. As oral iron ingestion is normally only 1–2 mg per day, it is

obvious that regular blood donation may be associated with iron depletion and results in a high occurrence of iron deficiency among blood donors<sup>5,6,7</sup>. Iron depletion not only cause iron deficiency anemia, but has also been related with physical and psychological disorders<sup>8,9,10</sup>. It is important to measure not just evident anemia but also the latent iron deficiency (LID) which is iron deficiency without anemia (normal hemoglobin). The people with LID are more likely to develop iron

deficiency anemia in the weeks or months. In seemingly healthy group of people such as blood donors, it is very important to diagnose LID to predict and anticipate an impending 'obvious anemia'<sup>1,3</sup> There are various diagnostic tests used for the assessment of possible iron deficiency anemia include indicators of disrupted heme synthesis such as zinc protoporphyrin or free erythrocyte protoporphyrin, mature erythrocyte indices including MCH, MCV, and RDW, as well as markers of iron stores, uptake, and breakdown, which include serum ferritin, serum iron, transferrin saturation, and soluble transferrin receptor. Freshly available, some newer electronic cell counters have an inbuilt parameter for measuring Hb in reticulocytes called reticulocyte haemoglobin content (ChR) or reticulocyte haemoglobin equivalent (Ret-He) measured by haematology analyser manufactured by Siemens (Advia 120 and 2120) and Sysmex Corporation and Mindray 6200<sup>8,9</sup>, respectively. Another parameter immature reticulocyte fraction (IRF) was introduced to indicate the less mature reticulocyte fraction. The IRF represents the proportion of young reticulocytes with the highest RNA content<sup>8</sup> It is defined as the ratio of immature reticulocytes to the total number of reticulocytes. They are one of the newer parameters of automated hematology analyzers and is a sensitive measure of erythropoiesis. The normal reference range for IRF is from 3.0% -15.9% in males and 2.3% - 13.4% in females. IRF more than 5% is taken as bone marrow recovery. It can be used as a sensitive parameter in diagnosis of Latent Iron deficiency in Blood donors.<sup>8,11,12</sup> A prospective study was conducted with the primary objective of detecting LID by using Ret-He and Immature Reticulocyte Fraction (IRF) in blood donors in comparison with Serum ferritin.

## Methodology

It is a prospective cross-sectional study conducted in department of Pathology and Blood Bank,

Pakistan Atomic Energy Commission Hospital (PAEC) Islamabad in collaboration with Blood Bank of Pakistan Institute of Medical sciences (PIMS) Islamabad. The study was conducted over a period of 6 months from July 2023 to December 2023. The age range of participants varied from 18 years to 60 years and included both male and female donors. Sample size was 192 which was calculated using WHO calculator. Sampling technique was consecutive nonprobability sampling. In all blood donors including new donors and regular blood donors, first they were registered at the front desk of the blood bank, and the donors were given predonation counselling by a Blood Transfusion officer. All the donors meeting the required criteria

## Results

The study was completed over a period of 6 months and sample size was 191 calculated by using WHO calculator. All donors were having normal Hemoglobin (males >13.0 gm/dl and females >12.0 gm/dl). Among 191 donors 157(82%) were males and rest 34 (18%) were females. according to WHO were asked for informed consent about the study, and those who provided consent were included in the study. Females having pregnancy, lactating or menstruating were not included in study. The blood sample was taken in Ethylene diamine tetraacetic acid (EDTA) vial and all extended red cell parameters including Reticulocytes indices (IRF and RHE) were measured using the Mindray 6200, fully automated haematology analyzer. The sample for serum ferritin was collected in clot activator vial in all donors to check Iron status. The study closely followed the moral standards set by PAEC ethical review committee. The data was entered and analyzed using SPSS version 20. The age range was between 22 to 50 years in our study. Most of the females 28(82.3%) were in age range of 22-30 years and males 65 (42%) were in 31-40 years' age group. Results are shown in the following tables.

**Table I: Shows statistics (mean, standard deviation, normal range) values of classical, conventional and newer iron investigations**

Parameters	Mean	Standard deviation	Normal Range
Age	36.35	8.12	18 to 60 years
<b>Classical Investigations</b>			
Hemoglobin (gm/dl)	14.40	1.08	F: 12.0-16.0 M: 13.0 - 16.5
MCV (fl)	90.09	7.74	77-93 fl
MCH (pg)	29.66	2.71	27-32 pg
MCHC (g/dl)	32.76	1.39	>30 g/dl
<b>Conventional Investigation</b>			
Serum Ferritin(ng/ml)	58.30	39.85	> 20ng/ml
<b>Newer Iron Investigations</b>			
Ret-Hemoglobin (RHE)	26.83	2.64	>26pg
IRF (%)	2.49	2.46	2.3 - 13.2%

**Table II: Cross table Analysis between Serum Ferritin and RHE**

	Ferritin		Total
	< 20	> 20	
<b>RHE</b>			
< 26	28 (14.66%) True Positive	10 (5.24%) False Positive	38 (19.90%)
>= 26	4 (2.09%) False Negative	149 (78.01%) True Negative	153 (80.10%)
<b>Sensitivity of RHE</b>	87.5%	<b>Specificity of RHE</b>	93.7%
<b>Total</b>	32 (16.75%)	159 (83.25%)	191 (100%)

Difference was statistically significant (P = 0.0001)] Ret-He >26 pg (normal value); Ret-He<26 pg (iron deficient), s Ferritin > 20 ng/ml (normal value); s

Ferritin <20 ng/ml (iron deficient]. Out of 191 blood donors in 32 donors' Ferritin were diagnosed with LID and 159 donors were having normal iron stores. Ret-He correctly diagnosed LID in 28 donors and missed it in 4. Similarly, of 159 donors suggestive of normal iron stores, Ret-He correctly diagnosed it in 149 cases and missed only in 10 of them.

**Table III: Cross table Analysis between Serum Ferritin and IRF**

	Ferritin		Total
	< 20	> 20	
<b>IRF</b>			
> 3	22 (11.52%) True Positive	21 (10.99%) False Positive	43 (22.51%)
<= 3	10 (5.24%) False Negative	138 (72.25%) True Negative	148 (77.49%)
<b>Sensitivity of IRF</b>	68.7%	<b>Specificity of IRF</b>	86.7%
<b>Total</b>	32 (16.76%)	159 (83.24%)	191 (100%)

Difference was statistically significant (P = 0.0001)] IRF < 3.0 (normal value); IRF >3.0 (iron deficient), s Ferritin > 20 ng/ml (normal value); s Ferritin <20 ng/ml (iron deficient]. Out of 191 blood donors in 32 donors' Ferritin were diagnosed with LID and 159 donors were having normal iron stores. IRD correctly diagnosed LID in 22 donors and missed it in 10. Similarly, of 159 donors suggestive of normal iron stores, IRF correctly diagnosed it in 138 cases and missed only in 21 of them.

## Discussion

Although the awareness about blood donation has increased many folds over the past few years<sup>9</sup>, the number of women donors is very less in our country because of several reasons such as cultural barriers and presence of anemia among females.<sup>10,13,14</sup> In the present study also, the number of female donors

was few (18%) as compared to male donors (82%). It was more than our principal study in which females' donors are 3% as compared to males (97%).<sup>1</sup> The mean age of donor population was  $36.35 \pm 8.12$  years among both male and female donors. The minimum and maximum age among the study group was 22 and 50 years, respectively. The donor age group was almost similar as compared to other studies on blood donors conducted by Tiwari et al.<sup>1</sup>, Shah et al. and Mahida et al.<sup>15,16</sup> on blood donors. No correlation was found between occurrence of LID and age of donor. In the present study Serum Ferritin diagnosed Latent Iron deficiency (LID) in 16.75 % donors. Ret-He detected LID among 14.6 % donors and failed to detect deficiency in 2.09%. The correlation between Serum Ferritin and RHE showed true positives (14.6%), true negatives (78.2%), sensitivity of RHE 87.5% and specificity 91.75%. the results are comparable to another study conducted by Tiwari et al. Which showed sensitivity of RHE 91.2% and specificity of 97.2% in concordance with their gold standard s TfR. Their P value was also <0.000 comparable to our study.<sup>1</sup> IRF correlated with s Ferritin in detecting LID among donors. In our study sensitivity of IRF in relation with Ferritin was 68.7% and specificity was 86.7%. A previous study by Nitika suria et al. The sensitivity of IRF was 45.1% specificity 93.4% The results obtained were in concordance with other studies conducted earlier.<sup>2</sup>

## Conclusion

Ret-He and IRF can be used as routine tests along with complete Blood picture in blood donors and could provide a chance to make appropriate interventions such as dietetic changes or iron therapy in them and it will not add to further cost.

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