Section: Pathology

Original Research Article

DETERMINATION OF REFERENCE RANGE FOR IMMATURE RETICULOCYTE FRACTION (IRF) IN HEALTHY ADULTS AND CORRELATION OF HAEMOGLOBIN WITH IRF: A CORRELATIVE STUDY

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Received : 15/06/2025
Received in revised form : 01/08/2025
Accepted : 19/08/2025

Keywords:
Anemia, Reticulocyte, Immature reticulocyte fraction, Automated, Beckman coulter LH750, hematology analyzer, transfusion.

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DOI: 10.47009/jamp.2025.7.4.252

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (4); 1328-1330

ABSTRACT

Background: Immature Reticulocyte Fraction (IRF) is useful in assessing the bone marrow's response to anemia. In this study, we aim to determine the reference ranges for IRF in healthy individuals on hematology analyzer, group anemias based on IRF reference range and correlate hemoglobin with IRF levels in proliferative and hypo-proliferative anemias. **Materials and Methods:** This study included 100 controls and 150 cases. Clinical details of 150 cases were recorded. Using EDTA treated venous blood, hemoglobin and IRF values were obtained from the automated BECKMAN COULTER LH 750. Reference ranges were derived for IRF in males and females. Anemias were classified into proliferative, normal and hypo-proliferative groups using the IRF reference range. Correlation tests were used to find correlation between hemoglobin and IRF. **Result:** The reference ranges derived from 50 male and female controls for IRF were 0.15 - 0.39, and 0.13 - 0.39 respectively. Of 150 cases, 30 cases were grouped in proliferative group (IRF>0.4), 100 cases belonged to normal group (IRF within reference range), while 20 cases were grouped in hypo-proliferative group (IRF < 0.15 in males, < 0.13 in females). In proliferative anemias, IRF showed good negative correlation with Hb in males and females. In hypo-proliferative anemias, IRF showed fair negative correlation with Hb in males and poor in females. **Conclusion:** IRF may be used in clinical diagnosis and follow-up of anemic patients, as they negatively correlate with hemoglobin values. As the IRF values vary according to instruments and population, studies are required to standardize the reference ranges.

INTRODUCTION

Blood cell production is usually maintained at steady state levels. Stressful conditions like
acute anemia, increases the reticulocyte count in the peripheral blood due to increased erythropoietin stimulation of erythoid precursors in the bone marrow. Automated methods use fluorescence and light scatter methods to measure the different reticulocyte population.[1]

In light scatter method, Immature Reticulocyte Fraction is a ratio of total number of reticulocytes in the outermost regions to the total number of reticulocytes.[2] An increase in immature reticulocyte fraction (IRF) represents an increase in immature reticulocytes recently released from the marrow and therefore is a reliable indicator of current bone marrow function.[3] Different laboratories use different instruments to measure IRF levels. Due to the differences in methodology, standardisation has not been done. This study aims to determine reference range of immature reticulocyte fraction (IRF) in healthy males and females using BECKMAN COULTER LH 750 which uses light scatter method and to correlate hemoglobin and IRF levels in proliferative and hypo-proliferative anemias.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology at a tertiary medical college in southern India. Approval of the ethics committee was taken prior to the start of the study [FMMC/FMIEC/1405/2013]. This correlative study was done between November 2013 to August 2015. IRF levels of 50 male and female controls respectively with normal haemoglobin(Hb) levels

ISSN (O): 2687-5365; ISSN (P): 2753-6556

were tabulated to establish the IRF range. The study included 150 cases belonging to the age group of 18 to 75 years with Hb levels <10g/dL. History and clinical examination findings were also recorded. The EDTA treated venous blood specimens received in hematology section from out-patient and in-patient departments were used to measure Hb and IRF levels. The parameters obtained by BECKMAN COULTER LH 750 were tabulated into the Microsoft Excel spread sheet and analyzed. Patients who had been treated for anemia were excluded from the study. Frequency tables, mean and standard deviation were used to establish reference range for IRF. Correlation tests were used to find correlation between hemoglobin and IRF.

RESULTS

The normal range obtained for IRF in males was 0.15-0.39 and in females was 0.13-0.39.

Using IRF range, the cases were classified into hypo-proliferative, normal and proliferative groups as shown in table 1. Mean values and standard deviation of IRF in males and females are shown in table 2. The percentage of males in hypo-proliferative group were 15.7%, normal were 70% and proliferative were 14.28%. The percentage of females in hypo-proliferative group were 21.25%, normal were 66.25% and proliferative group were 14.28%. In proliferative anemias, IRF showed good negative correlation with Hb in males and females. In hypoproliferative anemias, IRF showed fair negative correlation with Hb in males and poor correlation in females as shown in table 3.

Table 1: Number of cases based on IRF categories

	Mean	Count	N%
IRF categories	Hypoproliferative	30	20%
	Normal	100	66.7%
	Proliferative	20	13.3%

Table 2: Mean values and standard deviation of IRF in males and females

Gender	IRF mean	SD	mean -2sd	mean +2sd
Males	0.2712	0.060496	0.150208	0.392192
Females	0.262	0.065776	0.130447	0.393553

Table 3: Hemoglobin correlation with IRF

Proliferative	**Males**	**IRF**	**Pearson correlation**	**-0.521**	**Good correlation**
			Sig. (2-tailed)	0.123	
			N	10	
	Females	**IRF**	**Pearson correlation**	**-0.417**	**Good correlation**
			Sig. (2-tailed)	0.230	
			N	10	
Hypoproliferative	**Males**	**IRF**	**Pearson correlation**	**-0.335**	**Fair correlation**
			Sig. (2-tailed)	0.313	
			N	11	
	Females	**IRF**	**Pearson correlation**	**-0.144**	**Poor correlation**
			Sig. (2-tailed)	0.580	
			N	17	

DISCUSSION

Benefits of automated methods include greater precision of the counts (by analyzing a much greater number of reticulocytes (more than 10,000), the statistical error is minimized), elimination of interobserver variability and subjectivity, reduced turnaround time and variability resulting from staining, dilution, and incubation are also strictly controlled.[4]

One of the automated reticulocyte parameters is (IRF) which indicates the less mature reticulocyte fraction. The IRF represents the proportion of young reticulocytes with the highest RNA content. It is defined as the ratio of immature reticulocytes to the total number of reticulocytes. They are larger, having the greatest light scatter properties due to the highest level of ribonucleic acid. It is a sensitive measure of erythropoiesis. IRF rises in cases of increased marrow erythropoiesis before the increment of reticulocyte count. Therefore, those cells were found to be the earliest indicator of marrow erythropoietic activity.[5]

In this study, majority of the male controls were in the age group of 40-50 years while 24% of female controls were in 60-70 years age group followed by 20% each in 31-40, 41-50 and 51-60 years. This was similar to studies done by Hove LV et al who took healthy population composed of 48 males and 45 females using CELL DYNE4000.[6] Bosche VD et al chose more number of controls comprising of males n= 142 and females n=175.[7]

The IRF range obtained for males and females was 0.15-0.39 and 0.13- 0.39 respectively. The IRF

ISSN (O): 2687-5365; ISSN (P): 2753-6556

obtained by Chang and Kass using Sysmex R-3000 was 0.044-0.228, by Van den Bossche et al using CELL-DYN 4000 was 0.14-0.35, by GdS- Simel-Emat using LH 750 was 0.19-0.43 and by Banfi et al using LH 750 was 0.19-0.42.[7,8,9,10] The variation in control ranges was due to difference in reticulocyte reagents used in various instruments and differences in data analyses algorithms used to derive IRF as well as higher degree of inter subject variation.[11]

Based on IRF, the cases were divided into 3 groups: hypo-proliferative group comprising of 20 cases, normal comprising of 100 cases, proliferative comprising of 30 cases. Proliferative cases in males and females had IRF >0.4. This value agreed with studies by Fourcade et al who found that regenerative anemias had IRF values > 0.4. The causes of proliferative anemias agreed with studies by Fourcade et al and classification published in BECKMAN COULTER LH 750 bulletin.[13] Hypo-proliferative cases in males had IRF range 0.04-0.14 Hypo-proliferative cases in females had IRF range 0.03-0.12. The causes of low IRF agreed with studies by Fourcade et al and BECKMAN COULTER 750 Bulletin.[12,13] Proliferative anemias in both males (-0.521, P=0.123) and females (-0.417,P=0.23) showed good negative correlation with IRF than hypo-proliferative anemias {males(-0.335, P-0.313) and females(-0.144,P = 0.58)}. In hypo-proliferative anemias too, the correlation was good in females but weaker than proliferative in males. These findings agree with studies by Geldard et al 3 who observed a stronger negative correlation between Hb levels and IRF (-0.464, P<0.001) than for Hb levels in acute anemia. This observation was also found for patients in chronic anemia group but was weaker with a negative correlation between Hb level and IRF (-0.23, P<0.01). This shows that IRF is closely linked to Hb level. This relationship is stronger with proliferative anemias due to greater stress borne by the marrow and increased red cell production response. Hypo-proliferative anemias develop adaptive mechanism, so are better tolerated. This agrees with studies by Fourcade et al, who showed that IRF had the strongest relation with Hb.[13]

CONCLUSION

This study shows that IRF is a reliable method of bone marrow response.

However, each laboratory needs to determine its reference intervals as there are differences like the instrument used and the population studied.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556