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ASSESSMENT OF IMMATURE RETICULOCYTE FRACTION (IRF) WITH ABSOLUTE RETICULOCYTE COUNT (ARC) AS A RESPONSE MARKER IN ANTEPARTUM PATIENTS WITH NUTRITIONAL ANEMIA

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ABSTRACT

Background: Peripheral blood reticulocyte count is an important index in the diagnosis, classification, and monitoring of anemic patients. After initiation of therapy, reticulocytosis develops within 3 to 7 days and peaks (8–10%) between the 8th and 10th day[1]. The IRF (Immature Reticulocyte Fraction) increases several days before reticulocyte count and is thus an early indicator of response to therapy. **Aim:** To study the response of therapy using IRF on day 3rd- 4th and day7th- 8th in antenatal patients with nutritional anemia. To correlate ARC (Absolute Reticulocyte Count) with IRF after therapy in antenatal patients with nutritional anemia. **Study design:** Prospective, analytical observational study. **Materials and Methods:** A total of 63 pregnant women with nutritional anemia were enrolled. Baseline samples for RBC indices, ARC and IRF were taken and treated with parental iron and Vit B12 injection accordingly. Baseline, 3rd - 4th and 7th – 8th day of therapy, values of hematological parameters along with ARC and IRF for predicting the response were determined. **Results:** The rise in mean ARC and IRF from baseline to day3/4 and day7/8 is significantly higher in responders. There was a significant positive correlation between IRF and ARC at baseline, day3/4 and day7/8. **Conclusion:** IRF and ARC increase in nutritional anemia in subjects, as early as 3-4 days post-therapy, however ARC on day 3-4 is an accurate prediction of response to therapy as evaluated on day 7-day 8 in the present study.

KEYWORDS : Immature reticulocyte fraction. Absolute reticulocyte count. Early response marker.

INTRODUCTION

India has reported 87% prevalence of anemia in pregnancy[2]. Overall 40% of maternal deaths in the third world are related to anemia and among various causes of anemia 90% are nutritional in origin[3]. An important index in the diagnosis, classification, and monitoring of anemic patients is the peripheral blood reticulocyte count, which is a primary hematological test used to evaluate the bone marrow response to treatment for anemia.

IRF is an early and sensitive index of erythropoiesis that can be assessed on the 2nd-3rd day of hematinic therapy in deficiency anemias. Response to therapy is assessed by reticulocyte count on the 7-10th day. Reticulocyte count increases to 5-15%[4].

Aims & Objectives

- To study the response of therapy using immature reticulocyte fraction on day 3rd- 4th and day7th- 8th in antenatal patients with nutritional anemia.
- To correlate ARC (Absolute Reticulocyte Count) with IRF (Immature Reticulocyte Fraction) after therapy in antenatal patients with nutritional anemia.

MATERIALS AND METHODS:

The study was a prospective, analytical study conducted in the Department of Obstetrics and Gynecology, from September 2017 to May 2019, Hindu Rao Hospital, NDMC Medical College. Antenatal women with nutritional anemia attending OPD and inpatients were included in study.

A total of 63 pregnant women with nutritional anemia after informed written consent and fulfilling the inclusion and exclusion criteria were enrolled in the study. In the study group socio-demographic history, details of present pregnancy/ obstetric, menstrual, personal, and blood loss/transfusion related and dietary history were obtained. Detailed general, physical and systemic examinations were done.

Baseline hematological parameters (Hb, RBC, Hematocrit, MCV, MCH, MCHC, RDW, and Reticulocyte percentage) along with ARC and IRF were determined. Depending on the RBC indices and peripheral blood smear patients were categorized into microcytic, macrocytic or dimorphic anemia. Patients with peripheral blood smear and RBC indices suggestive of microcytic anemia and low serum iron and ferritin levels, low transferrin saturation were given iron therapy, oral or parenteral depending upon gestational age and severity of anemia.

Therapeutic dose of oral iron 100–200 mg elemental iron a day was given in two to three divided doses (ferrous sulfate is the oral preparation of choice)[5] parenteral therapy with iron Sucrose (Ferri S,) 200 mg i.v. injection every alternate day three doses per week depending upon the deficit in hemoglobin. Patients who are not compliant and not able to tolerate oral therapy are given parenteral therapy. Patient with the macrocytic picture given a dose of 1000mcg of parenteral cyanocobalamine along with 1mg of folic acid/day[6]. Patients with the dimorphic picture were given oral iron (ferrous sulfate) and folic acid and parenteral cyanocobalamine. Dietary counseling was done for all the patients. On the 3rd - 4th and 7th – 8th day of therapy values of hematological parameters along with ARC and IRF for predicting the response was determined. The cases were categorized based on rise in reticulocyte count. Patients with the rise in reticulocyte percentage <5% were labeled as nonresponders and >5% as responders.

The absolute reticulocyte count and fractions of HFR (High Fluorescence Ratio), MFR (Medium fluorescence ratio), and LFR (Low fluorescence ratio) were determined by Sysmex xt-4000; IRF is calculated as the sum of HFR and MFR (IRF=HFR+MFR)

RESULTS

There was no statistically significant difference between the two groups concerning demographic data.

The majority of the women in our study had moderate anemia 79.3%. There was no statistically significant difference between the distribution of patients according to the severity of anemia in the two groups.

Among nonresponders, 33% were those who were given parenteral therapy when compared to 69% of responders. Among nonresponders, 67% were those who were given oral therapy when compared to 31% of responders and this difference was statistically significant (p value=0.025).

Table 1: Comparison of mean hematological parameters between responders and nonresponders

Days \ Parameter		Hb	MCV	MCH	MCHC	RDW
Baseline	Responder	8.16 + 1.14	80.991 + 16.16	24.76 + 5.74	28.54 + 2.39	21.63 + 4.03
	Non Responder	8.64 + 1.09	78.35+ 10.76	23.37 + 3.49	28.69 + 2.09	20.19 + 8.25
	P Value	0.101	0.439	0.236	0.791	0.437
Day3/4	Responder	8.49 + 1.1	82.943 + 15.76	25.30 + 5.45	28.61 + 1.86	21.54 + 3.59
	Non Responder	8.88+ 1.07	79.40 + 10.69	23.89 +3.46	29.34 + 2.60	20.10 + 3.72
	P Value	0.174	0.293	0.211	0.167	0.139
Day 7/8	Responder	8.74+ 1.13	84.691 + 12.87	26.70 + 4.35	29.14 +2.13	22.70 + 4.91
	Non Responder	9.09+ 1.12	80.44 +8.85	25.11 + 3.31	29.85 + 2.12	21.40 + 4.37
	P Value	0.245	0.126	0.108	0.206	0.282

The difference in mean hemoglobin, RBC count, hematocrit, MCV/MCH/MCHC and RDW between responders and nonresponders at the baseline, day 3/4 and day7/8 were statistically insignificant (Table 1).

Table 2: Comparison of mean Reticulocyte %, ARC, and IRF between responders & nonresponders

		Retic % (Percentage)			ARC			IRF		
		NR	R	P	NR	R	P	NR	R	P
Baseline		2.62+ 1.35	3.07+ 1.07	0.173	93.99 ± 49.00	104.67+ 48.87	0.407	13.73 ± 7.89	16.83 + 7.00	0.123
Day3/4		2.81+ 1.65	5.60+ 1.86	0	98.17 ± 50.00	187.38+ 64.48	0.00	15.24+ 7.58	22.48 + 8.04	0.001
Day7/8		3.10+ 0.99	6.83+ 1.30	0	112.0 ± 42.43	235.06+ 60.63	0.00	16.72+ 9.20	20.50 + 9.24	0.122
p value	B vs 3/4	0.273	0.00		0.446	0.00		0.12	0.007	
	Bvs 7/8	0.015	0.00		0.014	0.00		0.03	0.051	

R – Responders NR – Non-Responders P – P value

The rise in mean reticulocyte percentage from baseline to day3/4 and day7/8 is significantly higher in responders(p-value=0.00). The rise from baseline to day3/4 is statistically insignificant in nonresponders but a significant rise is seen from baseline to day7/8 (p-value=0.0).

The rise in mean ARC from baseline to day3/4 and day7/8 is significantly higher in responders (p-value=0.00). The rise from baseline to day3/4 is statistically insignificant in nonresponders but a significant rise is seen from baseline to day7/8 (p-value=0.014) using the paired-samples t-test.

The rise in mean IRF from baseline to day3/4 and day7/8 is significantly higher in responders (p-value=0.007 and 0.051 respectively). The rise from baseline to day3/4 is statistically insignificant (p-value=0.12) in nonresponders but a significant rise is seen from baseline to day7/8 (p-value=0.03) using the paired-samples t-test.

Table 3: Correlation of IRF with ARF.

Correlation of IRF with ARF	P value	Coefficient(r)
Baseline	0.033	.269*
Day 3	0.006	.342**
Day 7	0.032	.270*

There was a significant positive correlation between IRF and ARC at baseline, day3/4 and day7/8.

*.Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed). P value – Pearson correlation.

A classification tree analysis was done and the suggested cut-off value for ARC was 127×109 /L and IRF was 19.3%. The cut-off value of 127×10^9/L for ARC on day 3, identifies responders on day 7 with a sensitivity of 86.95% and specificity of 85.05%, and overall accuracy of 85%. The cut-off value 19.3% for IRF on day 3, identifies responders on day 7/8 with a sensitivity of 69.56%, specificity of 72.50%, and overall accuracy of 71.42%. (Table 4)

Table 4: Distribution of cases according to the cut-off value of ARC and IRF.

		Actual result					
		ARC (x 109/ / L)		Total	IRF (%)		Total
		<127	>127		<19.3	>19.3	
Predicted	Non-Responders	34	3	37	29	7	36
Result	Responders	6	20	26	11	16	27
	Total	40	23	63	40	23	63

ROC curve analysis was done for ARC and IRF and the area under curves are 0.874 & 0.738 respectively.

Table 5: Evaluation of ARC and IRF on day 3/4 for identification of responders on day 7/8.

	ARC	IRF
Specificity	85.05%	72.50%
Sensitivity	86.95%	69.56%
Positive predictive value	76.92%	59.25%
Negative predictive value	91.89%	80.55%
Accuracy	85%	71.42%
Area under curve	0.874	0.738

DISCUSSION

The difference between the two groups concerning the age of the patients, antenatal booking status, socio-economic status, religion, dietary preferences, BMI & treatment given before inclusion in the study was statistically insignificant.

In our study, 78.3% pregnant women were in the 21-29 age group similar to **Rajamouli et al**[7] (77.3%, 20-29 years) and comprising various social status groups, categorized based on family income, found that most females from low income category were more iron deficient, most of them were Hindus (81%) and were vegetarians (66.6%) similar to **Sharma et al** [8], **Gautam et al**[9], and **Rajamouli et al**[7] respectively.

Good nutrition and adequate calories are essential for pregnant women, a lack of which might have led to anemia in the majority of our patients who had BMI <18 (23.8%).

In our study, 39.68% of women were booked and the remaining were either registered or unbooked cases indicating that anemia is more prevalent in those who had irregular or no antenatal visits and 61.9% were multi gravida similar to **Rajamouli et al**[7] and had high prevalence (79.3%) of

moderate anemia comparison to other degrees of anemia similar to **Goutham V et al**[9].

In our study, 53% were given oral therapy while 47% were given parenteral therapy (34%-Injection iron sucrose,13%-injection B-12 along with folic acid). Among responders, 69% were those who were given parenteral therapy and 67% of nonresponders were those who were given oral therapy i.e in cases given parenteral therapy responders are more. Despite ensuring the compliance of oral iron to our best, the increased gastrointestinal side effects might have caused compromised compliance in some patients and also, erratic and poor absorption of oral iron and other dietary factors might have led to not so good response to oral iron.

There was no significant difference between responders and nonresponders in mean hemoglobin, RBC count, hematocrit, and RBC indices at baseline, day3/4, and day7/8. But there was a statistically significant difference in mean reticulocyte percentage, IRF, and ARC at baseline, day 3/4 and day 7/8 respectively The significant increase in ARC was similar to **bruganara et al**[10] where there was a consistent increase in absolute reticulocyte count and CHr (Reticulocyte Haemoglobin content) suggesting that these measures of response to treatment may be reliable soon after treatment is started. **Parodi et al**[11] Data revealed a higher probability of being a complete early responder due to a relative increase of ARC from baseline to day7.

The significant increase in the IRF was similar to **Sudhir et al**[12] which shows that both reticulocyte hemoglobin and immature reticulocyte fraction are raised as early as 48 hours after iron therapy**. Goncalo et al**[13] observed that IRF recovery was anticipated by 4 days, IPF by 2 days after allogenic PB progenitor cell transplantation and concluded that new parameters IRF and IPF predicted hematopoietic recovery.

There was a significant increase in ARC and IRF after therapy similar to **Chang CC et al**[14], where increased IRF and increased ARC generally indicated an adequate erythroid response to anemia.

There was a significant positive correlation between ARC and IRF at the baseline, day 3/4, day7/8 in corroboration with **Chang cc et al**[14] We found that using cut off value of ARC $127\times10^{9}/\mu L$ suggested by classification tree analysis with sensitivity86.95%, specificity of 85.05% has a better accuracy of 85% when compared to IRF with accuracy of 71.42%, with a suggested cut-off value of 19.3%, its sensitivity and specificity are 69.56% and 72.5% respectively. ROC curve analysis was done and the area under the curve was greater for ARC compared to IRF

CONCLUSION:

IRF as well as ARC increases in nutritional anemia in subjects, as early as 3-4 days post-therapy, however ARC on day 3 - 4 is an accurate prediction of response to therapy as evaluated on day 7-8 in the present study.

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