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Clinical Application of Reticulocyte Maturity Indices in Early Diagnosis of Iron Deficiency Anemia in First Trimester of Pregnancy

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ABSTRACT

Objective: To thoroughly assess the diagnostic utility of reticulocyte maturity indices (RMIs) in iron deficiency anemia cases.

Methodology: This cross-sectional study was carried out from July 2023 to December 2023 at the Pakistan Institute of Medical Sciences (PIMS) and the Pakistan Atomic Energy Commission (PAEC) General Hospital, Islamabad. The laboratory studies included reticulocyte counts, hemoglobin and serum ferritin assays, and other parameters related to red blood cells. These assessments were used to divide the participants into different iron status groups. To identify differences in hematological parameters between the different iron status groups, statistical analysis was conducted using SPSS version 25 in accordance with ethical guidelines.

Results: Among 340 first-trimester pregnant women, three iron status groups were identified. Group 1 (n=102) had low ferritin despite normal Hb, with elevated immature reticulocytes. Group 2 (n=150) showed normal iron parameters, while Group 3 (n=88) had high reticulocyte counts. Group 2 exhibited superior erythrocyte indices and higher ferritin levels than Groups 1 and 3. Group 3 displayed the poorest indices and elevated immature reticulocytes. These findings highlight variations in iron status and reticulocyte maturation among pregnant women.

Conclusion: According to the findings of our investigation, reticulocyte hemoglobin content (HFR) and mean reticulocyte volume (MFR) are higher in pregnant women with iron deficiency anemia (IDA), which may indicate increased erythropoietic activity. These indices provide a rapid, efficient, and minimally invasive way to identify IDA in its early stages, which may be beneficial for clinical practice.

Keywords: Iron deficiency anemia, High fluorescence ratio, medium fluorescence ratio, Reticulocyte maturity indices

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [2,3] Critical analysis and manuscript review; [5,6] Data analysis; Manuscript Editing.

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Introduction

Iron deficiency anemia (IDA) is a prevalent hematological disorder globally, with a particularly high incidence among women of reproductive age, especially during pregnancy[1]. The first trimester of pregnancy presents a critical period when the demand for iron escalates due to the expansion of maternal blood volume and the rapid development of the fetal-placental unit.[2] Consequently, the occurrence of IDA during this phase can have profound implications for both maternal and fetal health. Aiming for a 50% reduction in anemia among women of reproductive age by 2025, the World Health Organization has set forth a global nutrition

target.[3] In 2013, iron deficiency anemia (IDA) emerged as the leading cause of anemia-related disability, with one in four pregnancies in Europe affected by it.[4] Globally, the estimated prevalence of anemia among pregnant women in 2011 stood at 38%, affecting approximately 32 million individuals. Additionally, the prevalence of iron deficiency in the absence of anemia ranges from 30 to 60%.[5] As erythropoiesis faces increasing compromise, iron stores progress from replete to diminished and eventually depleted, leading to anemia. Both anemic and non-anemic iron deficiencies during pregnancy bear repercussions for both the mother and her offspring.[6]Long-term impairments in cognitive development and growth have been observed in babies exposed to IDA in utero. Consequently, the prevention and treatment of anemia and iron deficiency in pregnancy are paramount.[7] Conventionally, the diagnosis and monitoring of IDA have relied on measuring parameters such as hemoglobin levels, hematocrit, and serum ferritin concentrations.[8] However, these traditional markers may not always provide a comprehensive assessment of erythropoiesis and iron utilization. Recent advancements in laboratory techniques have paved the way for exploring more nuanced indicators of erythrocyte production and maturation, with a focus on reticulocyte maturity indices.[9]

Reticulocytes, as immature red blood cells released from the bone marrow into the bloodstream, undergo a maturation process characterized by the loss of ribosomal RNA content before becoming fully functional erythrocytes.[10] The assessment of reticulocyte maturity indices, including the percentage of reticulocytes, mean corpuscular volume of reticulocytes (MCVr), and reticulocyte hemoglobin content (CHr), offers valuable insights into erythropoietic activity and iron availability.[11] In the context of IDA among first-trimester females, investigating reticulocyte maturity indices holds significant promise for enhancing our understanding of the underlying pathophysiology and optimizing diagnostic strategies. By elucidating the dynamics of erythrocyte maturation and hemoglobin synthesis, these indices may facilitate earlier detection of iron deficiency and monitoring of therapeutic interventions.[12] Moreover, incorporating reticulocyte maturity indices into clinical practice could aid in individualizing treatment approaches and improving outcomes for pregnant women with IDA.[13]

As a reliable substitute for manual reticulocyte counts, Automated Reticulocyte Analysis (ARA) has become increasingly popular in clinical laboratories in recent years. ARA provides improved efficiency and accuracy through the use of flow cytometry, which makes it a useful tool for tracking bone marrow recovery and diagnosing a variety of pathologies. This is especially important when a woman is pregnant, since her body goes through unique changes that make it difficult to determine her iron status and erythropoietic activity.[14] With ARA capabilities, a lot of contemporary automated haematology analyzers simplify diagnosis and speed up turnaround times. Reticulocytes can be divided into discrete subpopulations using ARA according to the intensity of their fluorescence, which is a direct indicator of their maturation state. Three main indices are produced by this classification: the Medium Fluorescence Ratio (MFR), the High Fluorescence Ratio (HFR), and the Low Fluorescence Ratio (LFR). These indices provide important information about the level of reticulocyte maturation; higher fluorescence intensity indicates lower maturity because of higher RNA content.[15]

Using ARA during pregnancy holds particular promise because iron deficiency anemia (IDA) presents serious risks to both the health of the mother and the fetus. Because ARA offers complete reticulocyte indices, prompt intervention can be facilitated by early detection and management of IDA during the first trimester, which is crucial for avoiding unfavorable outcomes. Personalized and targeted interventions to improve maternal and

foetal health outcomes can be made possible by healthcare providers evaluating the maturation profile of reticulocytes to spot subtle changes that point to an early iron deficiency before overt anemia manifests.[16]

The goal of this research is to thoroughly assess the diagnostic utility of reticulocyte maturity indices (RMIs) in iron deficiency anemia cases. This study attempts to precisely determine the diagnostic efficacy of reticulocyte maturity indices (RMIs) in identifying iron deficiency anemia through a detailed examination of Ret-He (reticulocyte hemoglobin content) and other relevant markers.

Methodology

This cross-sectional study was carried out in Islamabad, Pakistan, from July 2023 to December 2023 at the Pakistan Institute of Medical Sciences (PIMS) and the Pakistan Atomic Energy Commission (PAEC) General Hospital. All participants provided informed consent prior to inclusion in order to guarantee adherence to ethical standards and respect for personal autonomy.

The study included pregnant females in their first trimester, defined as up to 12 weeks of gestation, who were willing to undergo blood tests for the assessment of iron status and related hematological parameters. Exclusion criteria comprised pregnant females with known hematological disorders, a history of chronic illnesses affecting iron metabolism, or currently receiving iron supplementation or other treatments impacting iron levels.

During the data collection process, each participant's age, gestational age, and medical history were recorded. Blood samples were obtained for laboratory analysis, which involved the use of automated haematology analyzers to measure haemoglobin (Hb) levels and chemiluminescent immunoassay or enzyme-linked immunosorbent assay (ELISA) techniques to determine serum ferritin levels. Using flow cytometry, reticulocyte counts and reticulocyte indices (low, medium, and high fluorescence ratios) were evaluated. Standard laboratory procedures were used to measure additional red blood cell parameters, such as the total red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and red cell distribution width.

Participants were classified into three iron status groups based on the assessment of Hb and serum ferritin levels: Group 1 comprised pregnant females with normal Hb but low serum ferritin levels; Group 2 consisted of those with normal Hb and normal serum ferritin levels; and Group 3 included individuals exhibiting abnormal reticulocyte indices suggestive of iron deficiency.

Statistical analysis was conducted using SPSS 25.0 version, with descriptive statistics employed to summarize demographic characteristics and laboratory findings. Comparative analysis between groups was performed using suitable statistical tests (ANOVA, t-tests, chi-square tests), with p-values <0.05 considered statistically significant.

Ethical considerations adhered to principles outlined in the Declaration of Helsinki, with ethical approval obtained from the Institutional Review Board (IRB). Data analysis and interpretation aimed to elucidate differences in red blood cell parameters, serum ferritin levels, and reticulocyte indices among the identified iron status groups, providing insights into the prevalence and characteristics of iron status in pregnant females during the first trimester.

Results

Out of 340 pregnant women in their first trimester, three distinct iron status groups were identified. Group 1 (n=102) exhibited normal hemoglobin (Hb) levels but low serum ferritin, with elevated levels of

high fluorescence ratio (HFR) and medium fluorescence ratio (MFR) reticulocytes.

Table 1: Red blood cell parameters, serum ferritin, and reticulocyte indices in the three groups:

Parameters	Group 1 (n=102)	Group 2 (n=150)	Group 3 (n=88)	p-value
Total Red Blood Cell Count (TRBC, x 10^9/L)	4.5 ± 0.3	4.8 ± 0.4	4.2 ± 0.5	<0.001
Hemoglobin (Hb, g/dL)	11.2 ± 0.8	12.5 ± 0.7	10.8 ± 0.9	<0.001
Hematocrit (Hct, %)	35.5 ± 1.2	36.9 ± 1.1	35.1 ± 1.3	<0.001
Mean Corpuscular Hemoglobin (MCH, pg)	26.5 ± 1.1	29.8 ± 1.2	25.7 ± 1.3	<0.001
Mean Corpuscular Volume (MCV, fL)	78.2 ± 2.0	80.6 ± 1.8	77.4 ± 2.2	<0.001
Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL)	31.4 ± 0.8	32.7 ± 0.7	31.1 ± 0.9	<0.001
Red Cell Distribution Width (RDW, %)	13.8 ± 0.6	13.2 ± 0.5	14.0 ± 0.7	<0.001
Serum Ferritin (ng/mL)	20.3 ± 5.2	65.9 ± 10.5	8.6 ± 3.7	<0.001
Reticulocytes (%)	1.2 ± 0.2	1.0 ± 0.1	1.4 ± 0.3	<0.001
Low Fluorescence Ratio (LFR, % total reticulocytes)	87.2 ± 3.5	93.4 ± 2.7	84.9 ± 3.8	<0.001
Medium Fluorescence Ratio (MFR, % total reticulocytes)	9.3 ± 1.8	5.8 ± 1.2	11.2 ± 1.6	<0.001
High Fluorescence Ratio (HFR, % total reticulocytes)	3.5 ± 0.7	0.8 ± 0.3	3.9 ± 0.8	<0.001

Group 2 (n=150) had normal Hb and ferritin levels, and Group 3 (n=88) displayed high frequency reticulocytes and medium frequency reticulocytes.

Comparative analysis of red blood cell parameters, serum ferritin, and reticulocyte indices revealed significant differences among the three groups. Group 2 demonstrated superior erythrocyte indices with higher mean values of total red blood cell count (TRBC), Hb, hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) compared to Group 1 and Group 3 ($p < 0.001$ for all parameters). Group 3 exhibited the poorest erythrocyte indices with the lowest mean values of these parameters. Moreover, Group 2 had significantly higher serum ferritin levels than Group 1 and Group 3 ($p < 0.001$), indicating better iron stores. Conversely, Group 1 had the lowest serum ferritin levels among the groups.

Table 2: Reticulocyte parameters in the three groups:

Parameters	Group 1 (n=102)	Group 2 (n=150)	Group 3 (n=88)	p-value
Reticulocytes (%)	1.2 ± 0.2	1.0 ± 0.1	1.4 ± 0.3	<0.001
Low Fluorescence Ratio (LFR, % total reticulocytes)	87.2 ± 3.5	93.4 ± 2.7	84.9 ± 3.8	<0.001
Medium Fluorescence Ratio (MFR, % total reticulocytes)	9.3 ± 1.8	5.8 ± 1.2	11.2 ± 1.6	<0.001
High Fluorescence Ratio (HFR, % total reticulocytes)	3.5 ± 0.7	0.8 ± 0.3	3.9 ± 0.8	<0.001

Regarding reticulocyte parameters, Group 3 had the highest mean values of reticulocytes, low fluorescence ratio (LFR), MFR, and HFR, indicating higher proportions of immature reticulocytes and lower proportions of mature reticulocytes

compared to the other groups ($p < 0.001$ for all parameters). Group 1 exhibited elevated levels of HFR and MFR reticulocytes compared to Group 2, suggesting impaired maturation of reticulocytes despite normal Hb levels. These findings underscore substantial variations in erythrocyte parameters, serum ferritin levels, and reticulocyte indices among pregnant women categorized into different iron status groups, highlighting the importance of comprehensive iron status assessment during pregnancy.

Discussion

There is a lot of promise for using reticulocyte maturity indexes in the first trimester of pregnancy to diagnose iron deficiency anaemia (IDA). The high fluorescence ratio (HFR), medium fluorescence ratio (MFR), and low fluorescence ratio (LFR) are reticulocyte maturity indices that can be used to identify iron deficiency in the early stages before overt anaemia shows symptoms. Important information about erythropoiesis can be gained from these indices. The three diagnostic criteria that are frequently used in clinical practice have a strong correlation with the diagnosis of IDA in Chinese adults with CHr 27.2 pg, according to ZHANG Hui Di's research. With a cut-off value of 27.2 pg, CHr has a high diagnostic efficiency that is necessary to identify IDA.[17] Reticulocyte maturity indices first represent the reticulocytes' maturation stage and then provide a dynamic assessment of erythropoietic activity. The proportion of reticulocyte subtypes typically varies as a result of the bone marrow's increased production of immature reticulocytes in response to iron deficiency. Specifically, reticulocyte maturation impairment is suggested by low LFR and high MFR and HFR reticulocyte counts, which is characteristic of early-stage iron deficiency. The study carried out by Ringoringo *et al.,* found that the incidence of ID and IDA in infants.[1-4] months was 10.9% and 58.6%, respectively. The Ret-He cut-off values for iron deficiency and IDA were 22.25 pg, 20.3 pg, 19.05 pg, and 17.55 pg at 1, 2, 3, and 4 months. The specificity was 94.4%, 95.0%, 96.9%, and 98.1%, while the sensitivity was 7.8%, 2.5%, 7.4%, and 8.2%. In contrast to the negative predictive values of 18.1%, 19.6%, 33.0%, and 53.6%, the positive predictive values were 85.7%, 66.7%, 83.3%, and 80.0%. Ret-He can detect iron deficiency and IDA in infants 1-4 months old with a high specificity and strong positive predictive value.[18]

The results of Jamnok *et al.,* imply that the Ret-He formula might be helpful for ID screening in regions where thalassemia is common. Of all the individuals, 50% carried the thalassemia gene and 25% had iron deficiency (ID). We developed and examined several mathematical formulas using the receiver operating characteristic (ROC) curve. The formula derived from Ret-He: (Ret-He/RDW-SD) × 10 was found to be the most accurate predictor of ID among participants, as indicated by the area under the curve (AUC) = 0.812. When this indicator was tested again in individuals whose thalassemia screening results were positive, an AUC of 0.874 suggested improved performance.[19] The findings of Aedh *et al*. The measurement of Ret-Hb provides an easily accessible predictive marker for iron deficiency (ID) and IDA, when combined with CBC parameters and indices. If the Ret-Hb cut-off is decreased, it may be more useful as an IDA screening parameter. Ret-Hb levels, with a cut-off value of 21.2 pg (a value below which indicates IDA), were found to be significantly lower in IDA patients than in non-anemic individuals.[20] Furthermore, the early detection of iron deficiency through the use of reticulocyte maturity indices allows for timely intervention and preventive measures. Early detection of iron deficiency in the first trimester allows doctors to initiate appropriate iron supplementation and dietary modifications promptly. Proactive measures can prevent the progression of iron deficiency and

reduce the likelihood of complications related to iron deficiency, such as preterm birth, low birth weight, and maternal morbidity, for both the mother and the fetus.

Auerbach concluded in his study that the data showed abnormally low RET-He values. By identifying iron-deficient states and the need for iron replacement, these values can be used to quickly obtain standard iron parameters. Additionally correlated with both baseline and variation in RET-He is the Hb response. Using the RET-He improves patient and physician convenience by providing information on the necessity or lack thereof of iron replacement, given the high prevalence of iron deficiency in the general population. Additionally, it permits the delivery of conclusive therapy on the day of the first appointment.[21]

Moreover, there are advantages to using reticulocyte maturity indices as opposed to more traditional measures of iron status such as haemoglobin levels and serum ferritin. Unlike ferritin, which can be influenced by factors unrelated to iron status, reticulocyte maturity indices provide an objective assessment of erythropoietic activity and iron utilisation. Moreover, reticulocyte indices can detect early alterations in iron status before variations in haemoglobin levels, allowing for timely intervention and the prevention of iron deficiency anaemia.

However, it is important to acknowledge the limitations of reticulocyte maturity indices in the identification of iron deficiency anaemia. Reticulocyte index variations can indicate an early-stage iron deficiency, but they can also be caused by other conditions such as inflammation, hemolytic disorders, and vitamin deficiencies. Thus, a thorough approach incorporating several iron status markers is necessary for the accurate diagnosis and treatment of iron deficiency anaemia in pregnant women. In summary, the application of reticulocyte maturity indices in clinical contexts shows promise as a helpful tool for early detection of iron deficiency anaemia in the developing embryo. By providing dynamic insights into erythropoietic activity and iron utilisation, reticular indices facilitate timely intervention and preventive measures to mitigate the adverse effects of iron deficiency on maternal and foetal health. Further research is required to validate the utility of reticulocyte maturity indices in routine clinical practice and explore their potential impact on the specific iron supplementation regimens of pregnant women.

Conclusion

The results of our study show that pregnant females with iron deficiency anemia (IDA) have elevated Mean Reticulocyte Volume (MFR) and Reticulocyte Hemoglobin Content (HFR), which suggests increased erythropoietic activity. These indices offer a quick, effective, and low-invasive method for diagnosing IDA early on, which may have advantages for clinical practice.

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