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A DESCRIPTIVE STUDY TO ASSESS THE UTILITY OF RETICULOCYTE HAEMOGLOBIN EQUIVALENT IN THE EVALUATION OF IRON DEFICIENCY STATUS IN NORTH WESTERN INDIA

Pathology

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ABSTRACT

Background: Iron deficiency is a major global health issue linked to anemia, particularly in developing countries.(1) Traditional diagnostic methods, such as serum iron and ferritin can be unreliable due to the influence of inflammation associated with infections, chronic diseases and immune disorders. In contrast, the reticulocyte hemoglobin equivalent (RET-He) offers a more accurate assessment of iron deficiency, as it reflects the hemoglobin content in reticulocytes and is not affected by inflammation.(2) We aimed to study how RET-He performs in diagnosing iron deficiency and iron deficiency anemia in comparison with serum iron profile. **Methodology:** This cross-sectional study was done in SMS Medical College, Jaipur. The complete blood count, RET-He, serum iron profile and clinical parameters of 243 patients with microcytic hypochromic anemia and dimorphic anemia were reviewed and RET-He levels were compared with serum iron profile parameters. **Results:** The study population had a mean age of 40.2 years, with a notable prevalence of participants aged 21-30 years (30%) and a majority being female (79%), reflecting increased iron needs during menstruation and pregnancy. Participants predominantly resided in rural areas (63.8%), indicating potential dietary deficiencies and limited healthcare access. Common symptoms included fatigue (27.6%) and weakness (17.7%). All anemic participants exhibited decreased RET-He, suggesting 100% sensitivity for diagnosing IDA, including cases of IDA with raised ESR and dimorphic anemia. RET-He showed strong correlations with key hematological parameters lie MCH and MCHC along with moderate positive correlations with traditional iron parameters such as serum iron and ferritin. **Conclusion:** The study strongly supported the utility of Ret-He as a valuable parameter in assessing iron status and diagnosing iron deficiency anemia.

KEYWORDS

Reticulocyte hemoglobin equivalent, reliable, inflammation, sensitivity, correlation, iron deficiency anemia.

INTRODUCTION:

Anaemia is a condition in which the haemoglobin concentration is lower than normal limits for that age and sex. Anaemia, particularly iron deficiency anaemia (IDA), is a significant public health issue in India, affecting around 52% of non-pregnant women of reproductive age and contributing to high maternal mortality rates.[1] Iron is essential for numerous bodily functions, including oxygen transport and cognitive development, and its deficiency can lead to serious health consequences across all age groups.[2] Diagnosis typically relies on various biochemical tests, but these can be influenced by inflammatory conditions, complicating the detection of iron deficiency. Recent advancements suggest that the reticulocyte haemoglobin equivalent (RET-He) test may offer a more reliable and timely diagnosis of IDA.[3] RET-He measures the iron content in reticulocytes, providing insight into iron availability for red blood cell production. As reticulocytes have shorter life span (1-2 days) than mature red blood cells (120 days), the measurement of Reticulocyte haemoglobin equivalent can identify iron deficiency before the changes in the red cell parameters become apparent. RET-He provides the exact estimate of functional iron available for erythropoiesis. Unlike traditional serum tests, RET-He is more stable assay as it is not affected by inflammation or acute phase reaction. The recent automated count devices such as Sysmex XN1000 can measure reticulocyte haemoglobin equivalent in the same sample used for complete blood count.[2,4] The aim of this study was to assess the utility of RET-He in the evaluation of iron deficiency status of the patients.

MATERIALS AND METHOD:

This descriptive cross-sectional study was conducted at the Advanced Haematology Lab, SMS Medical College & Hospitals, Jaipur, from March 2023 to June 2024. The study included patients over 12 years of age with microcytic hypochromic anaemia (hemoglobin <11 mg/dL, mean corpuscular volume <80 fL) and dimorphic anaemia excluding pregnant females and post-transfusion samples. A sample size of 243 was calculated based on prior studies. Elaborate informed consent was obtained from the patients or their attendants before the commencement of the study. Ethical clearance was duly obtained from the Institutional Ethics Committee.

Clinical data were gathered using a proforma, which included demographic information, dietary history, and treatment history. The complete blood count (CBC) and reticulocyte hemoglobin equivalent (RET-He)of these samples were analyzed using the Sysmex XN1000 automated hematology analyzer along with serum iron profile, which included serum ferritin, serum iron level, total iron binding capacity (TIBC) and transferrin saturation.

Data were analyzed to explore associations between RET-He and serum iron profiles, as well as haematological and clinical parameters. Qualitative data were expressed in percentages, while quantitative data were reported as means with standard deviations. Statistical significance was considered at p-value of < 0.05.

RESULTS:

The study included a diverse sample of participants, with a mean age of 40.2 years and a predominance of females (79%). The majority of participants resided in rural areas, indicating potential barriers to healthcare access and nutrition. Educational background significantly influenced health outcomes, with a large percentage of participants having limited education, potentially affecting their dietary practices and awareness of iron deficiency. Clinical presentations included common symptoms like fatigue (27.6%) and weakness (17.7%), consistent with anemia. Maximum 162 (66.7%) cases were of iron deficiency anaemia, followed by IDA with raised ESR 59 (24.3%) and dimorphic anaemia 22 (9%). Out of 143 female participants 24 (9.9%) females were pregnant.

Table 1: Demographic Distribution Of Participants.

Parameter	Frequency	Percentage
Age Group (21–30)	73	30.0%
Female	192	79.0%
Male	51	21.0%
Rural Residence	155	63.8%
Urban Residence	88	36.2%
No education	53	21.8%
Primary/Secondary education	92	37.8%

The various haematological parameters (haemoglobin, TRBC, haematocrit and red cell indices) and RET-He were observed to be decreased below the lower limit values.

The RDW and IRF were increased, suggesting IDA and marrow response to therapy respectively. RET-He showed moderate correlations were with serum ferritin ($r = 0.290$, $p < 0.001$), serum iron

($r = 0.303$, $p < 0.001$) and hemoglobin levels ($r = 0.584$, $p < 0.001$) along with strong correlations with MCH ($r = 0.661$, $p < 0.001$) and MCHC ($r = 0.699$, $p < 0.001$) further validating RET-He's role as a marker of cellular iron content. Negative correlations of RET-He with RDW ($r = -0.185$, $p = 0.004$) and immature reticulocyte fraction (IRF) ($r = -0.523$, $p < 0.001$) indicate that lower iron availability is associated with greater variability in red blood cell.

RET- He showed 100% sensitivity for diagnosing iron deficiency in cases of IDA with inflammation and dimorphic anemia, which potentially affected the performance of serum iron profile parameters as a diagnostic tool in our study.

Table 2: Sensitivity of RET-He compared to Serum iron, Serum ferritin, TIBC and Transferrin saturation

Variables	IDA	IDA with raised ESR	Dimorphic anaemia
Reduced RET-He	100%	100%	100%
Reduced Serum Iron	98.7%	70%	81.8%
Reduced Serum Ferritin	70.3%	32.2%	63.6%
Increased TIBC	66%	18.6%	36.4%
Reduced Transferrin Saturation	91.9%	57.6%	77.2%

DISCUSSION:

This study was aimed to assess the utility of RET-He in evaluating the iron deficiency status of patients. By correlating RET-He with other hematological and iron indices, this research aimed to explore the potential of RET-He as a sensitive and specific marker for iron deficiency, improving diagnosis and management strategies.

The study population had a mean age of 40.2 years. Majority of participants (30%) were between 21-30 years of age, which is comparable to the study by Uçar MA et al. (2019)[6] and KS Mouleeswaran et al. (2022)[7] with a mean age of 39.8 years and 48.6 years respectively. MZ Miah et al. (2023)[8] observed 36% of participants having age between 18-30 years. Majority of the participants were female (79%), which was also noticed by KS Mouleeswaran et al. (2022)[7] (68.3% female participants) and MZ Miah (2023)[8] (82.8% female participants), reflecting increased iron needs during menstruation and pregnancy. Participants predominantly resided in rural areas (63.8%), indicating potential dietary deficiencies and limited healthcare access. Common symptoms included fatigue (27.6%) and weakness (17.7%), highlighting the clinical significance of iron deficiency.

Decreased RET-He value in our study is congruent with the studies of Bakri NA et al (2020)[9] and Morsed Miah et al (2023)[8]. However Vanessa Neef et al (2021)[10] observed higher value of RET-He probably because their study population was limited to children only. The decreased serum ferritin and iron levels were seen in 60% (147 out of 243) and 90% (219 out of 243) participants respectively, however all participants showing decreased Ret-He, suggests that Ret-He might be a more sensitive marker for iron deficiency than serum iron levels.

Table 3: Comparison of sensitivity of RET-He, serum ferritin and serum iron

Sensitivity of	Study by Mouleeswaran et al.(2022)[7]	Our Study
RET-He in IDA	97.4%	100%
RET-He in IDA with raised ESR	100%	100%
RET-He in Dimorphic anaemia	100%	100%
Serum ferritin in IDA	98.7%	70.3%
Serum ferritin in IDA with raised ESR	38.2%	32.2%
Serum ferritin in Dimorphic anaemia	100%	63.3%
Serum iron in IDA	100%	98.7%
Serum iron in IDA with raised ESR	100%	70%
Serum iron in Dimorphic anaemia	100%	81.8%

All anemic participants exhibited decreased RET-He, suggesting 100% sensitivity for diagnosing IDA, including cases of IDA with raised ESR and dimorphic anemia. Presence of raised ESR and dimorphic anaemia affected the performance of serum ferritin and serum iron as diagnostic tools.

Our study found a moderate positive correlation between Ret-He and haemoglobin. This correlation is consistent with the physiological relationship between iron availability (reflected by Ret-He) and hemoglobin production. The study by Bakri NA et al. (2020)[9] also found a significant correlation between Ret-He and hemoglobin.

The strong positive correlations with MCV, MCH and MCHC are congruent with the result of Bakri NA et al (2020)[9] which is consistent with the known effects of iron deficiency on red cells. These strong correlations are expected, as Ret-He directly reflects the hemoglobin content of reticulocytes, which is closely related to MCH and MCHC. The strong correlations with MCH and MCHC support the use of Ret-He as a marker of cellular hemoglobin content and, by extension, iron availability for erythropoiesis. In contrast to the study of Bakri NA et al (2020)[9] the negative correlation with RDW was observed in our study which suggests that as iron availability (reflected by Ret-He) decreases, there is more variability in red cell size, which is characteristic of iron deficiency anemia.

The moderate positive correlations with serum iron and serum ferritin are consistent with expected decreased in serum iron and ferritin levels which are used for diagnosis of IDA. Correlation is not strong which may reflect the limitations of serum iron and ferritin as markers of iron status due influence of inflammation and other factors. The study by Bakri NA et al (2020)[9] observed a more positive correlation with serum iron and serum ferritin as their study did not account for IDA with inflammation and dimorphic anaemia. The negative correlation with TIBC is consistent with the expected increase in TIBC in iron deficiency. A weak negative correlation was also observed by Bakri NA et al (2020)[9] which is comparable to our study. RET-He showed a weak positive correlation with transferrin saturation. The study by Bakri NA et al (2020)[9] showed moderate positive correlation. But they did not include participants of IDA with inflammation and dimorphic anaemia, both of which can affect serum iron required to calculate transferrin saturation.

In our study the sensitivity of RET-He for diagnosing IDA in all participants was 100%. This is comparable to the studies of Ali Ucar et al. (2019)[6] (90.4%), P. Chinudomwong et al. (2020)[11](96%) and K.S. Mouleeswaran et al. (2022)[7] (97.4%).

Our present study strongly supports the utility of Ret-He as a valuable parameter in assessing iron status and diagnosing iron deficiency anemia. The strong correlations observed between Ret-He and other haematological parameters, underscore its potential as an early and reliable indicator of cellular iron availability. Ret-He demonstrated consistent performance across diverse populations and clinical scenarios, suggesting its broad applicability in clinical practice. Importantly, Ret-He showed potential value in the context of inflammation, where traditional iron parameters like ferritin may be less reliable. This characteristic could make Ret-He particularly useful in clinical scenarios where inflammation is common, such as chronic diseases or pregnancy.

Limitation: The study provides a snapshot of relationships between parameters but cannot assess changes over time and did not assess the utility of Ret-He in monitoring response to iron therapy.

CONCLUSION:

Ret-He is a valuable parameter in assessing iron status and diagnosing iron deficiency anemia (IDA). The strong positive correlations observed between Ret-He and other hematological parameters, particularly MCH and MCHC along with moderate positive correlations between RET-He and traditional iron parameters such as serum iron and ferritin, underscore its potential as an early and reliable indicator of cellular iron availability. Ret-He demonstrated consistent performance across diverse populations and clinical scenarios, suggesting its broad applicability in clinical practice.

Conflict Of Interest: There was no conflict of interest in the study undertaken.

Ethics Approval: Ethics Clearance was obtained from Institutional Ethics Committee – S.M.S. Medical College & Attached Hospitals, Jaipur.

Informed Consent: Written Informed Consent was obtained from all the patients or their attendants before the study.

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