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Detection of Iron Deficiency Using Reticulocyte Haemoglobin in Adult Patients Having Microcytic Hypochromic Anaemia With Normal or Raised Serum Ferritin

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Abstract

Objective: To detect iron deficiency using reticulocyte haemoglobin in patients with microcytic hypochromic anemia having normal or raised serum ferritin.
Methodology: This Cross-sectional study was conducted in department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from February 2023 to July 2023, included 323 patients who presented with microcytic hypochromic anemia having normal or raised serum ferritin. Blood samples were taken in an Ethylenediamine tetra-acetic acid (EDTA) anticoagulant tubes and Sysmex XN 1000 analyzer was used to measure RBC parameters including reticulocyte Haemoglobin (Ret-He). Chi-square test and Independent T-test were applied for qualitative variables and quantitative variables respectively. Pearson's correlation test was used to correlate reticulocyte hemoglobin with RBC parameters. P value < 0.05 was considered statistically significant.
Results: Current study showed that the reticulocyte Haemoglobin with a cutoff value of 29 pg (sensitivity of 94.63% and a specificity of 85.48%, p<0.0001) is an efficient and reliable indicator to detect iron deficiency in patients presenting with microcytic hypochromic blood picture with normal or raised serum ferritin (r = +0.217, p < 0.001).
Conclusion: Our study concluded that reticulocyte haemoglobin can be a valuable marker for detecting iron deficiency in adult patients with microcytic hypochromic anaemia, especially when serum ferritin levels are normal or elevated.
Keywords: Beta Thalassemia, Hemoglobinopathies, Carrier Screening, Genetic Counseling.

Introduction

Iron deficiency remains a prevalent health concern globally particularly in developing countries. According to World Health Organization (WHO) nearly two billion individuals are anaemic, about half of them have Iron deficiency anaemia and defines anaemia as Haemoglobin (Hb) level <11g/dl in pregnant women and children below 5 years of age, <12.0 g/dl in women and <13.0 g/dl in men.[1]

Red blood cell (RBC) indices such as haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) have an essential role in evaluation of the causes of anaemia. These indices have been traditionally used since Wintrobe developed his anaemia classification in 1932.[2]

Depending on mean corpuscular volume MCV, anaemia can be characterized as microcytic (MCV: <76 fl), normocytic (MCV: 76-96 fl), or macrocytic (MCV: >96 fl). Microcytic anaemia was frequently characterized as hypochromic based on mean corpuscular Haemoglobin (MCH; N:27-32pg) and peripheral smear examination.[3,4] As mature red blood cell parameters, these indices are ineffective to detect early symptoms of iron deficiency. Microcytic hypochromic blood picture is associated with Iron deficiency anaemia (IDA) and other conditions like anaemia of chronic disease (ACD), thalassemia and sideroblastic anaemia.[5,6]

The reference method for diagnosis of iron deficiency anaemia is bone marrow aspiration and Prussian blue staining but this is an invasive and painful procedure. Traditionally transferrin and serum ferritin (N: 15-150 ug/L in females and 15-200 ug/L in males) are used to assess iron deficiency but are acute phase reactants and

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affected by conditions like acute inflammation and malignancies.[7] In certain conditions like chronic inflammatory disease, malignancies and end stage renal disease normal or raised serum ferritin fail to detect concomitant iron deficiency due to ambiguous assessment of iron status.[8] In the quest of accurate and timely diagnosis especially early detection of iron deficiency, the focus has been shifted towards exploring reticulocyte haemoglobin which is rapid, cost effective and precise red cell parameter that serves as an early sensitive indicator for iron deficiency before anemia develops, directly measuring the iron content in reticulocytes.[9]

Since the life span of reticulocytes is just 1-2 days, the reticulocyte Haemoglobin content gives a snapshot of available iron for erythropoiesis. It's a crucial factor in indicating iron deficiency that occurs when the tissues cannot readily access iron for use in bone marrow. This allows detection of iron deficiency before changes in red cell parameters become apparent. Assessing iron status is also important for patients undergoing treatment with erythropoietic stimulating agents, as iron parameters are significant in predicting the functional response of the bone marrow.[10,11] Two methods can be employed to evaluate reticulocyte hemoglobin. The first is the initial approach, referred to as reticulocyte hemoglobin content (CHr), which is present on Siemens analyzers. The second method, known as reticulocyte hemoglobin equivalent (RET-He), was developed later and is accessible on Sysmex analyzers.[12]

This study was designed to detect the iron deficiency using reticulocyte haemoglobin in patients with microcytic hypochromic anemia having normal or raised serum ferritin. Due to the high specificity, reticulocyte haemoglobin is an accurate diagnostic tool for early detection of iron deficiency anaemia.

Methodology

This cross sectional study was conducted in department of Haematology Armed Forces Institute of Pathology (AFIP), Rawalpindi from February2023 to July 2023. Approval was obtained from the Institutional Review Board (IRB) under reference number FC-HEM22-23/READ-IRB/23/1983. After review of literature sample size of 323 was calculated using the World Health Organization (WHO) calculator. The confidence interval was set at 95%, with a margin of error of 05% and a prevalence rate of microcytic hypochromic anemia found in previous studies at 30%. Sampling was done using non-probability consecutive sampling technique.

Inclusion Criteria: Participants of both genders between ages from 18 to 60 years having low MCV, MCH and serum ferritin >15 ng/ml were included in this study.

Exclusion Criteria: Patient with history of recent blood transfusion and therapy with hematinics were excluded from this study.

Written informed consent was obtained from the patients prior to enrollment and their confidentiality was ensured. Peripheral blood sample was collected in EDTA anticoagulant tubes. Using Sysmex XN 1000 automated hematology analyzer EDTA samples were subjected for complete blood count analysis. Haemoglobin level (Hb), MCV, MCH, and Reticulocyte Haemoglobin were recorded for each patient. The peripheral smears stained with Leishman stain were examined for RBCs morphology and cell counter generated parameters were correlated. According to the recommendations of International council of standardization in Hematology (ICSH), findings of microcytosis and hypochromic were graded as mild (+), moderate (++), and severe (+++).[13]

Anaemia was defined, following the WHO criteria, by Haemoglobin level< 12g/dl in women, < 11g/dl in pregnant women and children < 5 years of age and < 13g/dl in men, MCV < 76fl, MCH <27pg, Serum ferritin<15 ng/mL and an optimum cutoff value for reticulocyte haemoglobin 29pg (N: 29-36pg) for IDA, were considered for this study. Information about the patients' age, gender, and Hb levels, as well as their demographic and diagnostic details were also collected.[3,4,11,14,15]

Data was analyzed using SPSS version 24 software. Descriptive statistics were used to calculate means ± standard deviation for continuous variables. Frequencies and percentages were calculated for categorical variables. Shapiro Wilk test was used check distribution of data. Chi-square test and Mann-Whitney Test were used to calculate difference between qualitative variables and quantitative variables respectively. Spearman's Correlation was used for correlating Reticulocyte Hemoglobin with different Parameters. A significance

level of less than 0.05 for the p-value was deemed significant.

Results

This study included 323 participants. Among them, 191 (59.1%) were male with a mean age of 31.53±13.96, and 132 (40.9%) were females with mean age of 31.17 ± 10.63. The descriptive statistics provided key information about the study population, including their age, hemoglobin, MCV, MCH, reticulocyte hemoglobin and serum ferritin levels as seen in Table-I.

Table I: Descriptive Statistics of Study Variables

Variables	Mean ± SD
Age	31.39 ± 12.69
Mean Corpuscular Volume	65.16 ± 6.73
Mean Corpuscular Hemoglobin	20.07 ± 2.92
Reticulocyte Hemoglobin	23.96 ± 4.99
Serum Ferritin Level	130.63 ± 119.04
Hemoglobin	9.967 ± 1.11

Patients were classified into two groups based on serum ferritin level and different hematological parameters were compared as shown in table-II. There was a statistically significant difference in reticulocyte haemoglobin, MCH, and MCV levels between the two groups (p<0.0001).

Table II: Characteristics of patients in Normal and Raised serum ferritin level.

	Serum Ferritin Groups		p- value
	Normal	Raised	
	Mean ± SD	Mean ± SD	
Age	31.53 ± 13.31	30.85 ± 10.04	0.451
MCV	64.36 ± 6.62	68.36 ± 6.49	<0.0001
MCH	19.77 ± 2.81	21.23 ± 3.04	<0.0001
Reticulocyte- He	22.67 ± 4.03	28.93 ± 5.21	<0.0001

Correlations between Reticulocyte Hemoglobin and various parameters were established as seen in Table-III. Serum ferritin and Reticulocyte Haemoglobin showed a statistically significant positive linear association, r = +0.217, p < 0.0001.

Table III: Correlations between Reticulocyte Hemoglobin and certain studied parameters.

Parameters	Reticulocyte Hemoglobin, pg	
	Correlation Coefficient	p-value
MCH	0.548	<0.0001
MCV	0.557	<0.0001
Serum Ferritin	0.217	<0.0001

The association between serum ferritin levels, reticulocyte hemoglobin, and RBC indices was established, as seen in Table-IV. Data was stratified by normal and raised categories of serum ferritin. A Chi-square test was performed to assess the significance of the relationship between serum ferritin and reticulocyte hemoglobin. Reticulocyte hemoglobin, at cutoff 29 pg, had a sensitivity of 94.63% and a specificity of 85.48%, p<0.0001.

Table IV: Correlations between Serum Ferritin and Reticulocyte Hemoglobin.

Serum Ferritin	Reticulocyte Hemoglobin		p-value
	Anaemic (>29)	Normal (≤29)	
Normal	247 (TP)	9 (FP)	<0.0001
Raised	14 (FN)	53 (TN)	

*Sensitivity= TP/(TP+FN)= 247/(247+14)*100=94.63 %*
*Specificity= TN/(TN+FP)= 53/(53+9)*100=85.48%*
*Positive Predictive Value= TP/(TP+FP)*100= 247/(247+9)= 96.48%*
*Negative Predictive Value= TN/(TN+FN)*100=53/(53+14)= 79.10%*
*Diagnostic Accuracy=(TP+TN)/All patients*100 = (247+53)/323=92.88%*

Discussion

Iron Deficiency Anaemia (IDA) is prevalent worldwide, particularly in developing countries, as one of the most frequent forms of nutritional anemia.[16] Several factors can contribute to anaemia among adults and the elderly, such as chronic illness, reduced bone marrow response, inflammation, chronic kidney disease, and a decreased proportion of bone marrow to adipose cells. It's important to diagnose anemia accurately and promptly to prevent its adverse effects.

Routinely performed laboratory tests like Hb, MCH, MCHC and MCV may not promptly identify iron deficiency anemia. Detection of iron deficiency anemia (IDA) or latent iron deficiency (LID) solely through a single biomarker is unusual. However, there is need for readily available screening indicators, and additional research on this subject is still needed worldwide.[17] The level of serum ferritin is a frequently requested test in primary and secondary healthcare settings. Low serum ferritin levels indicate reduced iron stores in the body.

Guidelines for managing patients with low serum ferritin and iron deficiency anemia are well-established in medical practice.[18] However, in certain situations, such as when patients have concurrent inflammatory disorders; serum ferritin levels may appear normal or elevated despite the absence of iron stores. In such cases, it becomes challenging to diagnose anemia solely based on high serum ferritin levels, calling for a more effective method of detecting anemia in these patients.[19]

Reticulocytes originate in the bone marrow and undergo maturation into mature erythrocytes within 48 hours, after which they appear in the bloodstream. Consequently, analyzing the hemoglobin content in reticulocytes through blood samples is beneficial for assessing and evaluating iron levels. This assessment provides an accurate reflection of iron levels.

Mean reticulocyte hemoglobin level was 23.35 ± 4.79 in our study. Mehta et al, discovered similar results to ours, stating that the average Ret-He level among anemic patients in India was 19.19 ± 4.02 pg.[20] Similarly, Rungngu et al[21] found that Indonesian children with IDA had an average Ret-He level of 25.84 ± 4.87 pg.

Our data indicate that the cutoff value of 29pg/ml of reticulocyte hemoglobin has 94.63 % sensitivity, 85.48% specificity, and 92.88% accuracy to diagnose the iron deficiency anemia in the study population with microcytic hypochromic blood picture having normal or raised serum ferritin. Kilio et al and Khan et al described the similar value in their studies.[11,15]

Our study also found a significant correlation between Ret-He and serum ferritin with the p value of 0.001 similar to Nassim et al[22] study with p value 0.012. This study delves into the intricate interplay between reticulocyte hemoglobin and iron deficiency in a subset of patients presenting with microcytic hypochromic anaemia having normal or raised serum ferritin. Reticulocyte hemoglobin has a potential advantage as it is not influenced by the fluctuations that can affect levels of serum iron and serum ferritin.

Limitation of the study: Further studies with larger sample size may be helpful to unravel the precise pivotal role of reticulocyte haemoglobin through scientific landscape. So, for more accurate results this study should be conducted on the large scale and for longer time period.

Conclusion

Our study concluded that among the myriad indicators, reticulocyte hemoglobin with cutoff value of less than 29pg can be a valuable marker for detecting iron deficiency within subset of adult patients presenting with microcytic hypochromic anemia, especially when serum ferritin levels are normal or elevated.

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