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Differential diagnosis of microcytosis hyperchromic anaemia in children, correlation between peripheral blood film, erythrocyte indices, bone marrow study, and serum iron studies

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Abstract
Background and objective: To determine the relative frequency of different etiologies of microcytic hypochromic anaemia. To establish a correlation between these values and iron profile readings, assuming there is a high level of sensitivity. In order to determine the red cell index that best detects the underlying cause of anaemia in the absence of iron values, particularly the red cell index with the highest sensitivity, it is necessary to examine the red cell distribution width.
Methods: In the investigation, a sample of 60 children aged 6 months to 12 years, diagnosed with microcytic hypochromic anaemia based on peripheral smear reports and complete blood hemograms, were selected through a random sampling method to examine the correlation between red cell indices and serum iron profile, assuming that these indices possess both sensitivity and specificity for the purpose of differential diagnosis.
Results: Among the 60 cases, 41 (82% of the total) were diagnosed with iron deficiency anemia (IDA), 8 (16%) affected by anemia of chronic disease, and only 1 (2%) had thalassemia major. The correlative investigation demonstrated that red cell distribution width (RDW) is the most sensitive diagnostic tool for iron deficiency anemia in the differential diagnosis of microcytic hypochromic anemia. A statistically significant connection was observed between the Recommended Dietary Weight (RDW) and blood iron profile. The sensitivity of the red cell formulas Sirdah, RBC count, was shown to be 100% in diagnosing IDA, followed by RDWI and Mentzer's index.
Conclusion: In the context of ACD cases, a cost-effective strategy involves establishing a correlation between clinical characteristics and conducting a CRP level assessment to validate the presence of an underlying chronic condition. If the anemia remains uncorrected following the therapy of the underlying condition, it may be necessary to conduct a serum iron test. All instances of ACD in our investigation exhibited increased levels of CRP, in addition to the clinical manifestations of underlying illnesses. In 6 out of 8 cases of ACD, the RDW was within the usual range. In the case of thalassemia major, the need for lifelong transfusions necessitates expensive procedures such as Hb electrophoresis to confirm the diagnosis.

Keywords: Microcytic hypochromic anaemia, iron deficiency anaemia, anaemia of chronic disease, thalassemia major, red cell distribution width, red cell formulas, serum iron profile

Introduction
Iron deficiency anemia is the primary cause of microcytic hypochromic anemias in children, with thalassemias, sideroblastic anemias, lead poisoning, and anemia of chronic disease being less prevalent. The global prevalence of anemia among children under the age of five is estimated to be 47%. India exhibits the highest prevalence of anemia among the various nations. Anemia impacts 80% of children under the age of two [1, 2, 3]. Anemia affects 70% of children in India aged 6 to 59 months. Hemoglobinopathies have a global prevalence of approximately 10% among infants annually. The necessity of conducting a study that examines the correlation between the sensitivity of different red cell indices obtained from automated cell counters, peripheral smear results, and red cell formulas with the iron profile of anemic children arises from the financial and technological limitations associated with conducting iron profile studies, particularly in developing countries such as India [4, 5, 6].

Materials and Methods

The study was conducted on a cohort of 60 participants who were accepted and selected at random from the Department of Biochemistry at Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India, between January 2017 and December 2017. After receiving informed consent, samples were collected in 2 ml EDTA tubes and 3 ml in a separate sterile container.

The parameters under investigation in this study encompass mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). A peripheral smear was employed to subtype anemia. A reticulocyte count was conducted in order to assess the erythropoietic response of the bone marrow and to rule out the existence of hemolytic anemia. Hemoglobin electrophoresis (HbF) was used to confirm the diagnosis of thalassemia. In order to assess the iron profile, including serum iron, serum ferritin, percent saturation of transferrin, and total iron binding capacity (TIBC) values, a sterile container was employed to extract serum [7, 8, 9].

Inclusion criteria

1. Individuals ranging in age from 6 months to 12 years.
2. The WHO cut off values for anaemic children.
3. According to the peripheral smear and red cell indices, there is evidence of microcytic hypochromia.

Exclusion criteria

1. Infants under the age of 6 months.
2. Pediatric patients diagnosed with macrocytic and normocytic anemias.
3. Smears exhibiting significantly increased reticulocyte numbers.

Result

Table 1: RBC parameters

	IDA	ACD
RBC count (x 10^6 µL)	4.12 (1.6-4.68)	4.1 (3.35-5.06)
Hemoglobin (g./dl)	9.5 (3.1-10.9)	9.5 (6.5-11.4)
Hematocrit (%)	36.25 (9.7-33.2)	29.65 (22.8-32.8)
MCV (fml)	71.42 (50.3-80)	72.41 (57.7-79.2)
MCH (pg)	22.34 (11.5-28.6)	25.64 (15.2-31.3)
MCHC (g/dl)	31.56 (19.6-38.7)	32.89 (26-34.8)
RDW (%)	32.56 (12.7-34.9)	17.68 (12.3-22.6)

Table 2: Grading of smears

	No. of smears	Percentage
Grade 1 +	16	27%
Grade 2 +	22	36%
Grade 3 +	12	20%
Grade 4 +	10	17%

Table 3: Type of cases

Case type	Cases	%
IDA	46	77%
ACD	12	20%
Thalassemia major	2	3%
Total	60	100%
Average	17.78	

Discussion

The current investigation utilized a sample size of 60 cases that were selected in a random manner. The red cell indices, namely hemoglobin, RBC count, MCV, MCH, and MCHC, were obtained using the SYSMEX 3-part analyzer. The serum iron profile, comprising serum iron, serum ferritin, total iron binding capacity, and percent saturation of transferring, was acquired from a privately-owned laboratory. This analysis utilized the reference values. A significant method for differentiating between microcytic hypochromic anemia and other disorders has been discovered as the serum iron profile. Emphasizing the significance lies in the observation of connections between the iron profile and numerous markers, including red blood cell count, hemoglobin levels, and red blood cell distribution. The study population consisted of individuals ranging in age from 6 months to 3 years. 50%. Jain *et al*. (2016) conducted a study whereby they observed a significant occurrence of anemia in individuals between the ages of 1 and 2 years, with an incidence rate of 59.9%. [10, 11, 12].

The age cohort that encountered the most significant influence in the ICMR Study of 1985 was also confined to the 1 to 3 year range.63%. Arnab *et al*. did a study in Nepal which revealed that the age group with the highest prevalence was between 1 and 6 years old. The study conducted by Roosy Aulakh *et al*. demonstrated a higher incidence rate among individuals aged 1-5 years. This study aligns with previous research in terms of the primary age cohort under investigation. There is a significant increase in the prevalence of anemia among children aged 6 months to 3 years. According to the results of the present study, it was observed that among a group of 50 youngsters, a higher proportion of men (64%) were involved in the activity compared to females (36%). This finding aligns with previous studies. According to the findings of Sunil *et al*., half of the participants in their study were male. Santosh *et al*. and Roosy Aulakh *et al*. have documented male participation rates of 60% and 70% in their respective studies.

The findings of our study align with previous studies conducted by Sazawal *et al*. and Santosh *et al*., since we collected measures of mean corpuscular volume (MCV), mean renal distribution width (RDW), and biochemical profile, including mean serum ferritin. The purpose of this analysis is to analyze the parameters in order to determine if there is a statistically significant difference between the mean values acquired in our inquiry and those from previous studies, which would affect the result of the study. The inter-laboratory differences in blood ferritin levels can be attributed to the intrinsic heterogeneity in reference values [13, 14, 15].

The laboratory reference value exhibited a range of 7 to 140 ng/ml during our examination.

A comparative analysis was conducted to assess the sensitivity and specificity of Red cell distribution width (RDW) in relation to previous research. Multiple studies have demonstrated a sensitivity rate ranging from 95% to 100% in diagnosing early iron insufficiency. A sensitivity rating of 95% and a specificity rate of 66% were obtained from the continuing investigation. The threshold value has been reduced.

The sensitivity has increased from 17.4 to 13.4, while the

specificity has decreased. The present investigation attained a specificity rate of 66%, which may be ascribed to the utilization of a rather low threshold value. However, within our nation, where iron deficiency anemia (IDA) is the predominant form of microcytic hypochromic anemia, it is deemed appropriate to opt for a lower threshold value. This choice is justified by its ability to detect a greater percentage of individuals with IDA, hence offering benefits in terms of attaining favorable long-term results [16, 17, 18]. However, a drawback occurs when the accuracy is compromised, as the list of iron deficiency anemia (IDA) would include a larger number of cases of anemia caused by chronic illnesses and specific cases of heterozygous α and β thalassemias, even if the red blood cell count (RDW) is within the normal range. To achieve a desirable state of balance, it is advisable to establish a threshold for RDW that surpasses 15%, as indicated by the research conducted by Sazawal *et al*. and Kim *et al*. [19, 20, 21].

Adopting this practice would ensure a greater level of sensitivity and narrow focus. Sezawal *et al*. argue that the implementation of cost-effective approaches is important in order to tackle the high costs associated with diagnostic tests for the prevailing kind of microcytic anemia, such as iron status markers. There is a possibility that the rise in RDW could occur prior to the decline in MCV. Furthermore, it has been determined by the researchers that RDW demonstrates the potential to be utilized in extensive sample populations as a screening instrument for the detection of iron deficiency anemia. It is advisable to adhere to a recommended threshold of 15% for red blood cell (RDW) count, while prioritizing the maintenance of hemoglobin levels below 10 g/dl. The findings of the study indicate a statistically significant inverse correlation between reference daily water (RDW) and parameters such as serum ferritin, serum transferrin, and % iron saturation. Red cell formulae, namely the thalassemia trait (sometimes referred to as the discriminant index), are commonly used to differentiate between IDA and Beta thalassemia. However, Sazawal *et al*. support the possibility of using these methods to forecast IDA. In the present investigation, the diagnostic precision for pediatric patients diagnosed with IDA was found to be 95%, aligning with the findings documented by Aysel *et al*. (91%) and Ehsani *et al*. (94%). The sensitivity of 95% was consistent with the findings reported in the aforementioned study. The index's increased sensitivity enables a more accurate forecast of a greater number of IDA cases. However, the absence of specificity shown in the current investigation can be attributed to the presence of a control group primarily consisting of anaemic people with ACD [22, 23].

On the other hand, the aforementioned research incorporated persons with β-Thalassemia trait as control subjects due to their inclusion in regions with a notable incidence of thalassemia trait cases. The equation's denominator represents the count of red blood cells. The study conducted by Aysel *et al*. revealed that the mean red blood cell count was determined to be 5.6+ or -4. The examination yielded a mean red blood cell count of 3.6 million. Thalassemia is characterized by the presence of either a normal or elevated red blood cell count in affected individuals. Based on the elevated red blood cell (RBC) counts, the Mentzer's index would exhibit a value below 13, indicating a notable degree of specificity in their investigation.

The current investigation revealed that a significant proportion of persons diagnosed with ACD (88%) exhibited reduced numbers of red blood cells (RBCs), leading to a Mentzer's index exceeding 13, indicating a decreased level of specificity. Furthermore, the current investigation unveiled that all instances of ACD had elevated levels of C-reactive protein (CRP), indicating a predictive correlation with anemia of chronic illness. Once again, the red blood cell count serves as the denominator for the computation. Nevertheless, it is important to mention that the diagnostic point for IDA exceeded 3.8. The investigation conducted by Aysel *et al*. demonstrated a sensitivity of around 72% and a specificity of 85%. The investigation yielded a sensitivity of 85.36% and a specificity of 11.11%. Ensuring adequate treatment for iron deficiency anemia in early newborns is of utmost importance. A comprehensive assessment of the child's clinical history, nutritional evaluation, physical examination, study of peripheral blood films, and measurement of red cell indices are often employed methods to achieve an accurate diagnosis.

In situations where there is uncertainty, it may be imperative to administer a therapeutic trial of iron in conjunction with nutritional guidance. This specific procedure is regarded as rational and devoid of any adverse repercussions, particularly in instances involving infections or a thalassemia trait. The evaluation of the thalassemia characteristic can be conducted subsequent to the administration of iron deficiency treatment. In the event that the hemoglobin concentration and red cell indices fail to return to their baseline levels, additional inquiry may be pursued [22, 23].

Conclusion

The main causes of microcytic hypochromic anemia are iron deficiency, thalassemias, anemia of chronic disease, and, in rare instances, sideroblastic anemia. The study found that iron deficiency anemia and anemia of chronic disease were more common than thalassemia or sideroblastic anemia. Automated hematology analyzers, such as the MCV and MCH, play a crucial role in the classification of anemia into distinct kinds, including microcytic hypochromic anemia and others. The renal distribution width (RDW) serves as an early predictor of iron inadequacy by operating as an indicator of anisocytosis. The variations in RDW occur prior to the changes in MCV. The present investigation ascertained that the RDW exhibited a notable level of sensitivity, albeit with a comparatively lower degree of specificity, in the early detection of iron shortage. There was a significant negative association seen between RDW and serum ferritin, serum transferrin, and serum iron levels, suggesting that an increase in iron levels after therapy results in a return of RDW to its initial level. As a result, the observed association exhibits statistical significance. The present study revealed that the red cell formulas, namely sirdah and RDWI, demonstrated a sensitivity of 100% but a comparatively lower level of specificity. The diminished specificity seen in the present study can be ascribed to the diminished red blood cell count.

The current work has noticed that the C-Reactive protein is useful in distinguishing between cases of iron deficiency and anemia in persons with chronic illnesses. One hundred the peripheral smear demonstrated its usefulness primarily

in cases of significant iron deficiency, as it enabled the identification of pencil cells. Nevertheless, the sensitivity of the test was observed to be significantly diminished in instances of mild and moderate iron deficiency. In the course of this inquiry, a solitary occurrence of thalassemia major was identified through the examination of distinctive facial attributes, the detection of target cells in the peripheral blood film, and the customary hemoglobin electrophoresis pattern. Therefore, within the framework of Iron deficiency anemias, the optimal and cost-effective strategy, considering the available resources in a resource-constrained setting, would be. The objective is to improve the sensitivity of case detection by implementing a low mean corpuscular volume (MCV) and an enhanced relative deviation (RDW) with a threshold value of 15 in locations characterized by high prevalence. The peripheral smear can be used to confirm the presence of microcytic hypochromic morphology and the presence of pencil cells in cases of increased severity.

Iron deficiency anemia can be detected by directly calculating several red cell indices. Ultimately, the performance of a reticulocyte count serves the dual purpose of ruling out the potential occurrence of hemolysis and assessing the response of the bone marrow subsequent to a 2-week experiment involving oral iron therapy. In order to exclude and identify anemia of chronic disease, the measurement of C-reactive protein (CRP) can be conducted in all instances of microcytic anemias. To effectively manage anemia and chronic illness, the recommended line of action is as follows: The manner in which the underlying infections and inflammatory processes manifest in clinical settings. The current conditions. In the present study, a considerable proportion of children exhibited symptoms consistent with juvenile rheumatoid arthritis, while a minority were diagnosed with renal disease. There has been an increase in the levels of Erythrocyte sedimentation Rate (ESR) and C-reactive protein (CRP).

Further investigations aimed at finding the root cause of the inflammatory disease and administering appropriate therapy. As part of the treatment, a peripheral smear and complete hemogram may be performed to evaluate the correction of anemia. Expensive investigations, such as the assessment of blood hepcidin and serum iron levels, should only be undertaken in the absence of any amelioration in anemia.

The detection and verification of thalassemia major in the context of thalassemias heavily depend on costly methods, such as hemoglobin electrophoresis. The rationale for adopting this strategy is supported by the morbidity associated with the condition, including the requirement for lifelong blood transfusion initiatives. The identification of thalassemia minor or trait cases can be first accomplished through the observation of elevated red blood cell count and a normal red blood cell distribution width (RDW) value, in addition to the utilization of certain metrics related to red blood cells. Nevertheless, there is a necessity for the advancement of more sophisticated and economically viable alternatives.

Conflict of interest

Nil.

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