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Original research article

Evaluation of Iron Deficiency Anemia by Automated Cell Counters and Its Substantiation with Serum Ferritin Levels in The Pediatric Age Group

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Abstract

Anemia in children is one of the major social health problems in India and in many parts of the world and prevalence rate of anemia is an important indicator of the nutritional status within the pediatric population.

There is an estimate that 25% of the world population is affected by Iron Deficiency, the population groups most affected being infants aged between 4 and 24 months, school-age children, female adolescents, pregnant women and nurturing mothers. Infants and young children have a high risk for developing iron deficiency because they have a high demand for iron during the period of rapid growth. Iron deficiency leading to anemia will manifest itself as a Microcytic Hypochromic Anemia. Microcytosis develops either prior to or along with any reduction in hemoglobin levels. Hence the incidental detection of an isolated low Mean Corpuscular Volume (MCV) result could indicate early Iron Deficiency that has not yet resulted in anemia. Measurement of Serum Iron, Total Iron Binding Capacity (TIBC) & Serum Ferritin has been adopted as a test for detecting Iron Deficiency. The present study is undertaken to evaluate the occurrence of Iron Deficiency Anemia among the pediatric age group (1-12 years) with due importance for assessing the Serum Ferritin levels.

Key words: Anemia, Iron Deficiency, Ferritin, Pediatric, Microcytosis

Introduction

Anemia in children is one of the major social health hitches in India and also in many parts of the world. The prevalence rate of anemia is a vital indicator of the nutritional status within the pediatric population.

Anemic children have reduced exercise capacity, slower rate of growth, impaired cognitive development and delayed wound healing. Also, they have higher mortality risk due to complications associated with malnutrition and infection.

The study of the etiopathogenesis of anemia in infancy and childhood has attracted wide attention in the recent years in India. Infants and young children are at a high risk for developing iron deficiency as they have an increased requirement for iron during the period of rapid growth and development.

As because most of the body's iron exist in the hemoglobin of red cells, anemia is one of the first signs of iron deficiency. Iron Deficiency leading to anemia is a continuum, and there are three recognized phases: pre-latent, latent and iron deficiency states. This will manifest itself as a Microcytic Hypochromic Anemia in the peripheral blood smears.

Prelatent Iron Deficiency: Refers to a reduction in iron stores without reduced serum iron levels. It is detected by measuring Serum Ferritin.

Latent Iron Deficiency: Occurs when reticuloendothelial macrophage iron stores are depleted. The Serum Iron Level drops and TIBC increases without a change in hematocrit. This stage may be detected by a routine check of fasting, early morning transferrin saturation. It is a condition in which iron stores are exhausted but the blood hemoglobin (Hb) level remains higher than the lower limit of normal. In this

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stage, biochemical abnormalities in iron metabolism are detected, specially decreased Transferrin Saturation.

The Development of Frank Anemia: When the hemoglobin level falls below the lower limit of normal range, frank anemia develops.

	Prelatent	Latent	Frank Anemia
Free Erythrocyte Prophyrins/ FEP	Normal	High	High
Hemoglobin	Normal	Normal	<8
MCV	Normal	Normal	Low
Serum ferritin	Decreased	Decreased	Decreased
Marrow iron	Reduced	Absent	Absent
Serum transferrin	Reduced	<12	<12
Transferrin saturation	Normal	<16%	<16%

Microcytosis develops either prior to or along with any reduction in hemoglobin levels. Hence, the incidental detection of an isolated low mean corpuscular volume (MCV) result could indicate early iron deficiency that has not yet resulted in anemia.

The available iron is measurable as serum iron and any excess iron in the body is stored in a nontoxic storage form or ferritin. Thereby, normal levels indicate adequate iron stores, thus, low levels of the same indicate iron deficiency state. Also, to be understood is that serum ferritin is widely recognized as an acute phase reactant and marker of acute and chronic inflammation, and is nonspecifically elevated in a wide range of inflammatory conditions besides being in iron overload state. And so, measurement of serum ferritin has been widely adopted as a test for iron deficiency and also iron overload.

Ferritin in human serum reflects body iron stores, measurement of serum ferritin has been widely adopted as a test for iron deficiency and iron overload [1]. Normal ferritin concentration varies by age and sex. Concentrations are high at birth, rise during the first two months of life and then fall throughout later infancy. At about one year of age, concentrations begin to rise again and continue to increase into adulthood [2].

The present study is undertaken to evaluate the occurrence of iron deficiency anemia among the pediatric age group (1-12 years) with due importance for assessing the serum ferritin levels.

Aims and Objectives

The objectives of the present study were to study the hematological parameters by automation, their association by study of the biochemical parameters including serum iron studies along with distinct reference to serum ferritin levels, the iron storage form, and the various changes in the peripheral smears in children of age group 1 to 12 years who are clinically suspected to have Iron Deficiency Anemia.

Materials and Methods

The present study is a cross sectional, descriptive study conducted on 100 children in the age group of 1 year to 12 years, having a clinical suspicion of anemia along with hemoglobin level of less than 11.5 gm/dl were included in the study who attended the pediatric department of a city based medical college and hospital of Kolkata after obtaining informed consent from the patient's guardian. The study duration has been for the duration of 20 months, i.e., August 2021 to March 2023.

However, children previously diagnosed as thalassemia, sideroblastic anemia, iron deficiency anemia or any other hematological diseases were exempted. Also, neonates were not taken into consideration.

A detailed clinical history was taken & a thorough clinical examination was undertaken. Venous blood samples were drawn from all children with proper aseptic precautions. Samples for hematological investigations were collected in EDTA vacutainers and for serum iron studies and ferritin assessment, in red capped vacutainers.

The hematological investigations were performed on a five-part cell counter and the parameters were considered for the study included hemoglobin, hematocrit, red cell total count, all red cell indices including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC). Also taken into account were Red Cell Distribution Width or RDW.

Anemia was categorized as

Anemia Status	Hemoglobin in 1-5 yrs of Age	Hemoglobin in 6-12 yrs of Age
ABSENT	>11.0 gm/dL	>11.5 gm/dL
MILD	10.0-10.9 gm/dL	11.0-11.4 gm/dL
MODERATE	7.0-9.9 gm/dL	8.0-10.9 gm/dL
SEVERE	<7.0 gm/dL	<8.0 gm/dL

Peripheral smears were prepared on glass slides and stained with Leishman's stain. They were examined by a light microscope and relevant observations were made upon the size of red blood cells. The red cell

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morphology was categorized as normocytic, microcytic and macrocytic depending on whether the RBCs were the size of a small lymphocyte or smaller. These were corroborated with the cell counter MCV values. When the MCV was less than 73fl among children between 1-5years & less than 75fl among children 4 to 11 years, the type was defined as microcytic. It was categorized as macrocytic when MCV was more than 100fl. The rest were put under the normocytic group. With anemia, if the red cell distribution was more than 15%, with predominantly microcytic red cells, the condition was grouped under Iron Deficiency Anemia (IDA).
Serum ferritin estimation was done on an automated biochemical machine.
In the present study, WHO criteria were used to define anemias. A hemoglobin level less than 11 gm/dl in children between 1-5yrs and less than 11.5 gm/dl in children between 6-12 years was the defining measures.
This was corroborated with the serum ferritin levels which were low and the cut off was less than 12 micro gm/dl

Observations and Results
The present study, conducted in the Department of Pathology, in a city based medical college and hospital, is a prospective, clinical non-controlled study of 100 children within the age group of 1 to 12 years who attended the OPD/IPD of Pediatrics Department from August 2021 to March 2023. Children found to have hemoglobin less than 11.5gm/dL were evaluated for anemia.

The findings have been tabulated as follows:

Table 1: Distribution of anemia in the two different genders

Gender	No of patients	Percentage
Female	28	28%
Male	72	72%
Total	100	100%

Table 2: Distribution of anemia in different pediatric age groups

Age Group	No of patients	Percentage
1-4 yrs	14	14%
5-8 yrs	38	38%
9-12 yrs	48	48%
Total	100	100%

Table 3: Distribution of morphologic anemia in the different age groups

Age group in years	Microcytic	Normocytic	Dimorphic
1-4	14	0	0
5-8	26	7	5
9-12	38	4	6
Total cases =100	78	11	11

Table 4: Distribution of anemia according to severity

Severity of Anemia	No of patients	Percentage
Mild	19	19%
Moderate	53	53%
Severe	28	28%
Total	100	100%

Table 5: Distribution of anemia according to severity in different age groups

Age Group	Severity of Anemia No. of patients			Total
	Mild	Moderate	Severe	
1-4 yrs	10	4	0	14
5-8 yrs	9	22	7	38
9-12 yrs	0	27	21	48

Table 6: Relation of Packed Cell Volume (PCV) with severity of anemia

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Severity of Anemia	PCV (normal: 30.2-42.3%)	
	Range	Mean
Mild (N=19)	31.9-84.8	37.29
Moderate (N=53)	19.5-33	27.01
Severe (N=28)	14.6-28	21.34

Table 7: Relation of RDW (Red cell distribution width)with severity of anemia

Severity of Anemia	RDW (normal =11.5 to 14.5)	
	Range	Mean
Mild (N=19)	12.4-23.5	15.54
Moderate (N=53)	12.6-27.6	17.73
Severe (N=28)	15.9-24	19.06

Table 8: Serum Ferritin Levels

Anemia	No. Of cases	Serum ferritin (Normal Range 12-400 µgm/mL)	Serum ferritin Mean
Mild	23	10.2-12	11.13
Moderate	40	7.2-9.6	8.14
Severe	37	4.1-7.0	6.04

Discussion

Pediatric Anemia is an important universal problem [3]. In India, anemia is the most common nutritional problem affecting more than half of the total population, particularly the children and the pregnant women[4]. Given the detrimental long-term effects and high prevalence of iron deficiency, its prevention in early childhood is an important public health issue [4].

During the last 2 decades, automated blood cell counters have undergone a formidable technological evolution owing to the introduction of new physical principles for cellular analysis. For some consolidated parameters, such as WBC and RBC counts, hemoglobin concentration and Mean Corpuscular Volume (MCV), analytic performance by the automated counters is generally excellent. From the RBC volume distribution histogram, modern analyzers calculate an index of heterogeneity known as the RDW, expressed as a percentage coefficient of variation and less frequently as the standard deviation.

In our present study, we included 100 children of age group between 1 to 12 years, whose hemoglobin was less than 11.5 gm/dL for 6-12 years children & less than 11gm/dL in the age group 1 to 5 years.

The different conclusions arrived at during this study include the following

Gender-wise distribution of anemia

The male child was found to be more vulnerable, though the gender difference could be because of the additional concern the Indian low socio-economic class has for the male child.

Table 9: Comparative study of gender wise distribution of anemias in the pediatric age group

Ratio	Present study	Gomber [5]
Male:Female	1:2.6	1:1.2

It was also noted that, more males were found to be anemic as compared to the females, 1:2.6. A similar gender distribution was noted in the study by Gomber *et al* [5].

Age group maximally affected

It was found that in the present study, that among the 100 cases studied, 53% had moderate anemia, 28% had severe anemia and 19% had mild anemia. And it was also noted that the children that were were maximally affected were in the age group of 9 years to 11years i.e., about 48%. Also in this age group, 53% had moderate anemia and 28% had severe anemia.

Using the Chi-square test, the association between the age group and the severity of anemia was found to be significant ($p<0.05$). It was also noted that the severity of anemia is significantly associated with the Packed Cell Volume ($p<0.001$).

RBC indices and RDW, valuable parameters which define the red cell morphology and hereby quick diagnosis of alleged iron deficiency anemias and so RBC indices score better over peripheral smear examination.

Microcytic Hypochromic Anemia (78%) was the most common type followed by Normocytic Hypochromic Anemia (11%) and Dimorphic Anemia (11%). This finding is in concurrence with the study by Kapur *et al* [6],

In the present study of 100 cases between the age group of 1-12 years, 70 cases were diagnosed to have iron deficiency anemia. Out of the 70 cases of iron deficiency anemia, 56 cases (80%) had decreased

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packed cell volume (PCV), 13 cases (18.5%) had a normal packed cell volume (PCV) and in 1 case (1.4%) the packed cell volume (PCV) was increased.
In this study of 100 cases, 73% had low MCV, 26% had normal MCV and in 1 case the MCV was increased.
The next hematological parameter that was taken into account was the Red Cell Distribution Width or the RDW. In our study the RDW was increased in 86% cases, decreased in 10% cases and was normal in 4% cases. The Mean RDW was comparatively high in children with severe anemia than those having mild to moderate anemia.
The relationship of RDW with severity of anemia was significant (p<0.001) using Analysis of Variance.
In our study, examination of stained peripheral smears revealed a Microcytic Hypochromic Blood Picture in 78% children. Thus, our study is in accordance with the study done by Beutler E [7]. Among the less severely anemic patients, normal red cell indices were common and examination of stained smears were not superior to determination of red cell indices. The examination of stained smears was important but could not exclude the diagnosis of iron deficiency anemia on the basis of normal appearance of red cells on smear examination alone.
In this study, we also came to the supposition similar to Jen P *et al* [8], regarding the peripheral smear examination. It could be stated that blood smear examination performed no better than automated RBC indices assessment in detecting probable iron deficiency anemia.
Serum ferritin is the most reliable biochemical parameter as anemia definitely has all morphological features of IDA when the iron storage form, serum ferritin is low.
In the present study undertaken, serum ferritin was less than 12 ug/l in 77% cases. So present study correlates with Thoradeniya [9] (74.2%), Zeben VD [10] (90%), Alper BS [11] (54%).
Jean Pinter *et al* [12] in their study concluded that the importance of serum ferritin level provides a clear separation between iron deficiency anemia (below 20ug/l) and thalassemia major (more than 500ug/ml).

Table 10: Percentage distribution of Serum Ferritin in IDA in comparison with other studies

Authors	Serum Ferritin (<12 microgram/l)
Thoradeniya [9]	74.2%
Zeben VD [10]	90%
Alper BS [11]	54%
Present Study	89.74%

A limitation to our study is that we did not use a gold standard to diagnose Iron Deficiency. Gold standard for the diagnosis of Iron Deficiency is a bone marrow biopsy or aspiration & stained by Prussian blue: Perl's method for iron stores. Given the invasiveness of obtaining a bone marrow specimen, evaluation of IDA by the hemoglobin concentration, RBC indices, and biochemical measures of iron deficiency is typically used for diagnosis of iron deficiency [13].
Difficulties were encountered in bringing the subjects back to the pediatric OPD for repeated laboratory evaluation after iron therapy, only few of them accomplished this goal, not enough for meaningful comparisons.

Conclusion

One of the most important areas for scope in the improvement of primary health care is the prevention of nutritional deficiency especially Iron Deficiency, because it has been associated with delay in psychomotor development and increased morbidity and mortality in children. Special need to be undertaken to educate the masses, so that the initial symptoms of iron deficiency anemia are not ignored and the children are brought to the hospital at the earliest for timely diagnosis and effective management.
Children being the most vulnerable group for Iron Deficiency Anemia, require early screening. Initial screening and subsequent diagnostic tests enable early diagnosis and appropriate management. Utilization of technologic advances is beneficial in arriving at a definitive diagnosis.
Since, in the present study, the school going children within the age group 9 to 12 years were found to be the most affected. Hence, it is recommended that, this age group is compulsorily screened for Iron Deficiency Anemia.

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