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APPLICABILITY OF ADOPTING MORPHOLOGICAL PATTERNS IN PEDIATRIC ANEMIAS AS ETIOLOGICAL INDICATORS

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ABSTRACT

Background: Pediatric anemia is a global phenomenon. The magnitude of this problem gets exaggerated in developing countries like India wherein majority of the population are vulnerable since they live in rural areas and come under low socio-economic status. Though pediatric anemias can be diagnosed by simple blood tests like complete hemogram and peripheral smear examination, the evaluation of underlying etiological factor to initiate appropriate therapy involves expensive work up which may not be affordable in a rural setup. This need-based study of pediatric anemia was conducted to determine the morphological patterns of anemia by simple baseline investigations and to detect the related etiologic factor. **Methodology**: The probable etiological factors contributing to the anemias were diagnosed by determining the morphological patterns using simple baseline investigations like complete hemogram and peripheral blood examination and treated accordingly. **Conclusion:** The etiology of microcytic hypochromic anemia was found out to be iron deficiency as confirmed by serum iron profile studies. Presence of dimorphic anemia suggested combined nutritional deficiency of Iron, vitamin B12 deficiency or folate. Hemolytic anemias detected by routine hematological investigations were confirmed by serum electrophoresis. Morphological examination of red blood cells on peripheral smear examination is thus an effective, simple and cost effective method to determine the etiology of various types of anemia.

KEYWORDS: Peripheral blood smear examination, Baseline investigation, Patient management.

INTRODUCTION

Anemia is defined as a reduction of the total circulating red cell mass below normal limits. Anemia reduces the oxygen-carrying capacity of the blood, leading to tissue hypoxia. Clinically useful approach classifies anemias according to alterations in red cell morphology. Etiology is determined by looking at morphology of RBC's which includes red cell size as normocytic, microcytic, or macrocytic, or the color of red cells as normochromic or hypochromic which determines degree of hemoglobinization. [1]

Normal ranges of Hemoglobin in pediatric age group. [2]

11.5-15.5 gm/dL 6-12 years

11.0-14.0 gm/dL 6 months -6 years

9.5-14.0 gm/dL 2 months -6 months

13.6-19.6 gm/dL Newborns

Anemia is a major health problem in India. In children, it assumes greater importance because of many adverse effects on their health including changes in immune functions, cognitive development, temperature regulation, energy metabolism and work performance. The situation in developing countries like India with her legacy of overpopulation, poverty, illiteracy, religious taboos and malnutrition is worse. More than 75% of Indian toddlers are anemic. The pathogenesis of anemia in children constitutes a syndrome of multiple etiology and varied presentations due to environmental factors and complex developmental processes. Among them, nutritional anemia is

most the common. Nutritional anemia refers to a condition in which the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency.[3] Anemia is a major nutritional problem worldwide and its prevalence is higher in developing countries when compared to the developed countries.[4,5] Anemia hinders the development of country by decreasing the working capacity as it affects the physical and mental development of an individual. [6] Iron deficiency anemia is a major health problem of children with adverse effects on their development. It is an insidious problem, unnoticed, often not diagnosed, yet it saps the vitality of the nation. In most cases, anemia can be diagnosed with a few simple blood tests. In addition, if the hemoglobin level is low, further evaluation is needed to determine the type of anemia present. A complete hemogram including peripheral blood examination will suffice to determine the morphological pattern of anemia. The evaluation of the underlying etiological factor involves expensive workup which is not feasible in a rural setup where majority of the patients hail from low socio-economic strata of society. Hence the basic peripheral smear examination which is a simple, quick, cost-effective and minimally invasive test to determine the morphological patterns of anemia provides a clue to the probable etiologic factor thus contributing to the effective patient management which is ideally suited to the needs of a rural tertiary care hospital.

MATERIALS AND METHODS

This prospective study was conducted on 70 patients in the age group of 0 – 18 years who were clinically suspected to have anemia. The children with low Hb were categorized as 5 groups. Uncooperative patients and those suffering from congenital heart diseases were excluded from the study.

After obtaining informed consent from either of the parents or guardian, blood samples were received or drawn in the pathology laboratory under aseptic precautions by venepuncture using a 2 ml syringe in EDTA coated containers from the clinically suspected cases of pediatric anemia. Complete clinical data including name, age, sex, presenting signs and symptoms were recorded.

The blood was run in automated three part cell counter for complete hemogram work-up.

Peripheral smears were prepared from the same sample and stained by Leishman's stain and examined under light microscope and the morphological patterns of anemia were determined. Special investigations like Hb electrophoresis, serum iron and vitamin B12 assays were conducted whenever required.

METHODOLOGY:

An initial estimation of Hb is done at the laboratory with the help of automated three part cell counter (Haematology analyser sysmex KX-21). If the Hb value thus obtained indicated anemia, a detailed history was elicited, a thorough clinical examination was done and the data was recorded in the proforma. Blood smears were prepared and stained with Leishman's stain and thoroughly examined under oil immersion objective of light microscope. The other parameters were assessed by complete hemogram.

RESULTS

After estimating Hb the subjects were categorized into five age groups, children aged between 6 months to 6 years were found to be the most affected thus constituting 71.4%, followed by 14.3% among the children aged between 6 years to 12 years, 8.3% among the children aged between 12 years to 18 years and 2.8% among the infants aged between 0 to 6 months. **(Table 1)** Their morphological patterns were determined by peripheral smear examination.

Table 1. Age wise distribution pattern of pediatric anemias.

Age group of patients	Number	%
0 – 2 months	2	2.8
2 months -6 months	2	2.8
6 months – 6 years	50	71.4
6 years – 12 years	10	14.3
12 years – 18 years	6	8.5
Total	70	100

Out of 70 cases studied, 50 (71.4%) were males and 20 (28.6%) were females. The male: female ratio in the present study was found to be 2.5:1. (Table 2)

Table 2.Genderwise distribution pattern of pediatric anemias

Gender	Number	%
Male	50	71.4
Female	20	28.6
Total	70	100

Anemia was found to be most prevalent in the age group of 6 months – 6 years wherein, 25 children had hemoglobin range between 1- 9.4 g/dl and 23 children had hemoglobin range between 9.5- 10.9g/dl. (Table 3)

Table 3. Agewise distribution patterns of hemoglobin percentage in different age groups of pediatric anemias

Age	Hemoglobin (in g/ dl)							Total
	1 - 9.4	9.5 - 10.9	11.0 - 11.4	11.5 - 13.5	13.6 - 13.9	14.0 - 15.4	15.5 - 19.6	
0 - 2 m	1	1	0	0	0	0	0	2
2 - 6 m	1	1	0	0	0	0	0	2
6m-6 y	25	23	2	0	0	0	0	50
6-12 y	7	1	2	0	0	0	0	10
12-18 y	3	2	1	0	0	0	0	6
Total	37	28	5	0	0	0	0	70

m: months, y: years

Microcytic hypochromic anemias are characterized by marked anisopoikilocytosis and predominance of microcytes [MCV<80 fl] and reflected by hypochromasia [MCHC<32g/dl]. It is usually seen in iron deficiency, thalassemias, sideroblastic anemia and anemia of chronic disease. In this study 44 cases of microcytic hypochromic anemia were reported and the etiology was confirmed by serum iron profile studies whenever feasible.

Presence of any two populations of red cells including microcytes, macrocytes or normocytes signifies dimorphic blood picture. 12 cases of dimorphic blood picture were encountered in the present study.

Normocytic normochromic blood picture is characterized by normocytes [MCV: 80-100fl] and decreased hematocrit and decreased hemoglobin value. It is usually seen in acute blood loss, hemolytic anemia, bone marrow failure and anemia of chronic disorder and vitamin deficiency. In this study 4 cases of normocytic normochromic anemias were reported.

Hemolytic anemia [Figure 1] is mainly caused due to abnormal breakdown of RBCs either in blood vessels or elsewhere in the human body. It is seen in hereditary spherocytosis, thalassemia, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency and pyruvate deficiency. In the present study, eight different types of hemolytic anemias were reported.

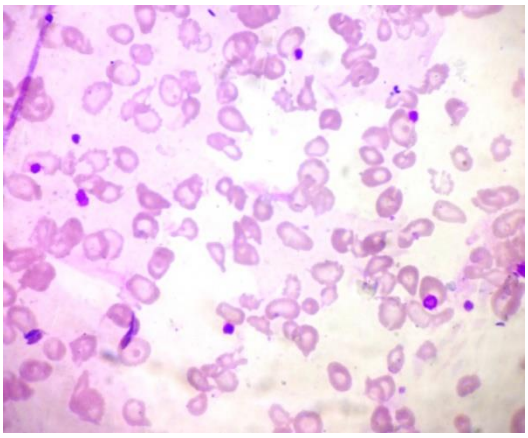

Figure 1. [Leishman stain- x 100x] Hemolytic blood picture showing crenated RBCs, normoblasts and polychromatophilic RBCs

Hereditary spherocytosis (HS) [Figure 2] is a non immune – mediated hemolytic anemia characterized by the production of spherocytes. In the present study, mild splenomegaly was observed in both the cases of HS. Moderate degree of anisocytosis, many microspherocytes and polychromatophilic red blood cells were seen in both the cases. Reticulocyte count was increased in both the cases(7.8 % and 8.4% respectively) .In one of the cases, direct Coomb's test was negative, total bilirubin was 0.4 mg/dl and direct bilirubin was 0.2 mg/dl. Liver function tests were within normal limits. In one of the cases, the grandfather was a known case of HS, while in the other, the mother was a known case of HS and had undergone splenectomy.

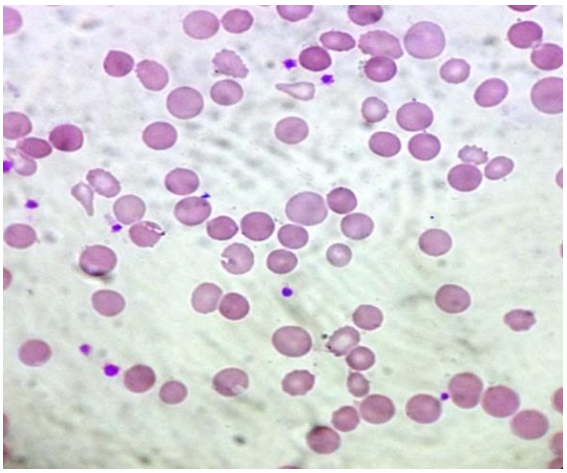

Figure 2. [Leishman stain- x 100x] Spherocytes in hereditary spherocytosis

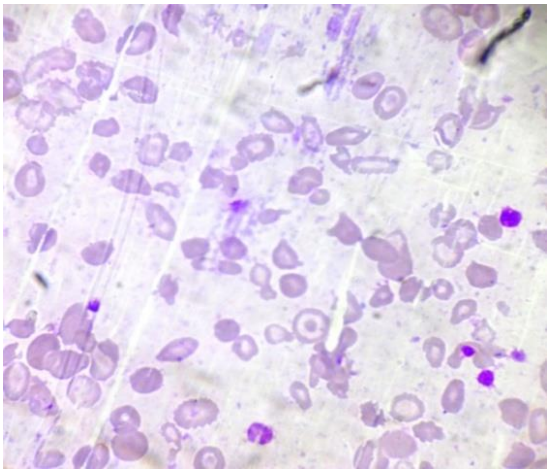

Figure 3. [Leishman stain- x 100x] Hemolytic blood picture in Hemoglobin D Punjab disease

One rare case of Hemoglobin D Punjab, [Figure 3] aHb variant, which occurs mainly in north west India, Pakistan and Iran was detected. Hb D Punjab or Los Angeles differs structurally from normal Hb A at 121 position on beta chain, where glutamine replaces glutamic acid. The child presented with severe pallor, depressed nasal bridge and hepatosplenomegaly. Serum electrophoresis was done for confirmation which showed increased levels of foetal hemoglobin (5.2%) Hemoglobin A0 and A2 were normal and Hb D was increased (36.7%). The patient's father had a macrocytic blood picture, mother had normocytic normochromic blood picture and the brother had a microcytic blood picture. LDH levels were also increased (4500 IU/L).The peripheral smear showed severe anisopoikilocytosis, predominant macrocytes and macroovalocytes. Occasional nucleated RBCs, red cells with basophilic stippling and features of hemolysis were seen.

One case of hemolytic disease of the newborn which is also known as erythroblastosis foetalis, a hemolytic disease caused by blood group incompatability between mother and fetus, was encountered in the present study. In this case, the blood group of the mother was O Rh negative and that of the foetus was O Rh positive. Peripheral smear showed features of hemolytic anemia with a good number of polychromatophilic red blood cells. Reticulocyte count was remarkably increased (20.7%). Indirect coomb's test was positive in the mother and direct coomb's test was positive in the child.

Four cases of β –thalassemias [Figure 4] were detected in the present study. There was no family history of thalassemia in any of the cases. All the four patients presented with pallor, icterus, hepatomegaly and splenomegaly. The peripheral smear findings included anisopoikilocytosis, increased number of polychromatophilic RBCs and normoblasts. Reticulocyte count was increased in all the cases. In β thalassemia minor, hemoglobin electrophoresis usually reveals an increase in HbA2. Hb F levels are generally normal or occasionally slightly increased. In the present study, serum electrophoresis was done for confirmation in all the four cases which showed markedly elevated Hb A2 levels and slightly elevated Hb F levels, thus confirming the diagnosis of β thalassemia minor.

Out of the forty four cases of microcytic hypochromic pattern detected on complete hemogram and peripheral smear examination, the iron deficiency was confirmed by serum iron profile studies whenever it was affordable to the patient. Serum iron levels were found to be decreased in these cases thus proving that iron deficiency was the underlying etiologic factor in microcytic hypochromic patterns. The eight cases in which hemolytic patterns were encountered on peripheral smear examination, were confirmed as hemolytic anemias and subtyped by hemoglobin electrophoresis. Serum electrophoresis is mandatory for confirmation of etiologic factor in hemolytic anemias and the morphologic pattern alone does not suffice. Confirmation of etiology was not possible in twelve cases with dimorphic blood picture due to non- concurrence by the patients

for bone marrow examination or serum iron/ vitamin B12/ folate studies.

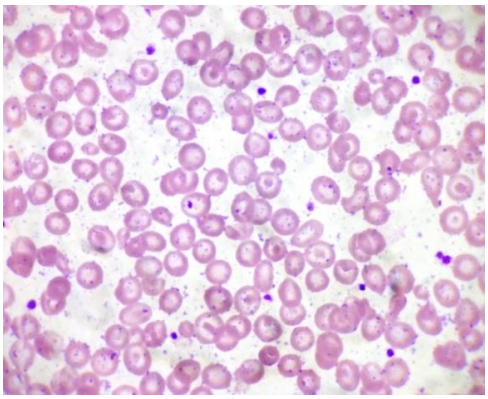

Figure 4. [Leishman stain- x 100x] Anisopoikilocytosis, polychromatophilic red cells and target cells in thalassemia

In the present study, the most common morphological pattern of anemia was microcytic hypochromic (62.8%) followed by dimorphic (17.1%), Hemolytic (11.4%) and normocytic normochromic (5.7%). Normocytic hypochromic pattern (2.9%) was the least common**.** Different types of hemolytic anemias were observed during the study. Some rare hemolytic anemias including, two cases of hereditary spherocytosis, one case of hemolytic disease of newborn, four cases of β thalassemia and one case of Hemoglobin D Punjab which is an exceptionally rare entity in this part of the world were encountered.

Table 4. Distribution of the various morphological patterns of pediatric anemias

Morphological types	Number	%
Microcytic hypochromic anemia	**44**	**62.8**
Normocytic hypochromic anemia	**2**	**2.9**
Normocytic normochromic anemia	**4**	**5.7**
Dimorphic anemia	**12**	**17.1**
Hemolytic anemia	**8**	**11.4**
Total	**70**	**100**

Relationship between morphological patterns of pediatric anemias and etiological factors in the present study

In the present study, in cases of microcytic hypochromic pattern detected on peripheral smear examination, the iron deficiency was confirmed by serum iron profile studies whenever it was affordable to the patient. Serum iron levels were found to be decreased in these cases thus proving that iron deficiency was the underlying etiologic factor in microcytic hypochromic patterns and that Serum iron assay is not mandatory if it is unaffordable to the patient. The clinician initiated iron therapy in these cases and the response to therapy was favorable. In all the cases of dimorphic blood picture detected on peripheral smear examination, serum assays for iron , Vitamin B12/Folate or bone marrow examination were not accessible due to the non-concurrence of the patients. Combination therapy was initiated. The cases in which hemolytic patterns were encountered on peripheral smear examination were confirmed as hemolytic anemias and subtyped by hemoglobin electrophoresis. Serum electrophoresis is mandatory for confirmation of the etiologic factor in hemolytic anemias and the morphologic pattern alone does not suffice to initiate treatment.

DISCUSSION

The mainstay of the present study was to diagnose pediatric anemias and to categorize them under thevarious morphological types and thereby determine the probable underlying etiologic factors in order to facilitatepatient management by adapting simple, minimally invasive and cost effective and baseline investigations.

Burden of anemia is alarmingly more as the World Health Organization (WHO) reports highest prevalence of anemia (47.4%) among preschool-aged children. [7] 80% of Indian children aged between 12 to 23 months are anemic according to the third National Family Health Survey (NFHS) 2005-2006. [8]

Gomber S et al in their study opined that childhood anemia is a significant public health problem in school children and that iron deficiency either alone or in combination is the commonest nutritional cause of anemia. They concluded that presence of vitamin B12 deficiency was the second most common cause of deficiency anemia and that pure or mixed vitamin B12 deficiency was an important yet not commonly recognized cause of anemia in school children aged between 5 and 11 years.[9]

Sindhu S et al estimated hemoglobin by cyanmethaemoglobin method and diagnosed anemia in their study. Out of the 1200

preschool Bazigar tribal children studied, only 9.50%were normal and 90.50% were affected with various grades of anaemic conditions, 6.33% being mildly anemic and 75.75% moderately anemic while 8.42% suffered from severe anemia. They found the cause to be higher dietary inadequacy of all the nutrients.[10]

Pasricha et al observed among 12- to 23-month-old rural Indian children that the hemoglobin levels in children were primarily related to iron stores, levels of folate, CRP and thalassemia trait. The hemoglobin levels were independently associated with maternal hemoglobin level, family wealth and food insecurity. They concluded that, combining iron supplementation and food-fortification programs with efforts to reduce maternal anemia, family poverty, and food insecurity help in combating the problem of pediatric anemias.[11]

Firdossaba et al conducted a study among the hospitalized children at a multispecialty hospital, Banglore, Karnataka to find the occurrence of anemia, the patterns of anemia, and its distribution in different age groups. They concluded that, the occurrence of anemia among children aged between 6 months and 12 years was high and nonhemoglobinopathies predominated over the hemoglobinopathies.[12]

Rathna S et al undertook a study hospital based prospective cross sectional study from March to May 2014which included hundred cases with hemoglobin less than 12g% and age group ranging from 6 months to 6 years. Results showed that the maximum numbers of the patients were in the range of 6 months to 1 year. Proportions of anemia in males was 55% when compared to females (45%). Normocytic normochromic anemia was the commonest type of anemia. Microcytic anemia was seen more frequently among female patients and normocytic normo/hypochromic anemia was commonly observed among male children. They concluded that the study of patterns of anemia is essential as it reflects the underlying etiopathological factors. [13]

In the present study, children within the age group 6 months to 6 years were maximally affected which is in concurrence with the study by Sindhu et al., whereas, in a study by Gomber et al and Sunil Pal Singh C children in the age group of 10-11 years were maximally affected. (Table 5)

Table 5. Comparative study of most affected age group in pediatric patients

	Age	%
Present study	6 months – 6 years	71.4%
Sindhu et al	2-3 years	94.55%
Sunil Pal Singh c	10-11 years	26.5%
Gomber et al	10.0-10.9 years	27.3%

In the present study, more number of male children were found to be anemic as compared to female children. A similar gender distribution was noted in the study by Gomber et al. Whereas, in a study conducted by Sunil Pal Singh C females were affected more than males. In the study conducted by Sindhu et al there was no difference in the gender distribution. (Table 6)

Table 6. Comparative study of genderwise distribution of pediatric anemias

	Male : female
Present study	2.5 :1
Sindhu et al	1.01:1
Gomber et al	1.16:1
Sunil Pal Singh c	0.8: 1

In the present study, microcytic hypochromic (62.8%) was the most common morphological pattern followed by dimorphic (17.1%), hemolytic (11.4%), normocytic normochromic (5.7%), normocytic hypochromic (2.9%). There was no incidence of macrocytic anemia in the present study which is in concurrence with the study by Kapur et al[14], wherein microcytic hypochromic anemia (27%) was found to be the most common followed by normocytic hypochromic anemia (27%), normocytic normochromic anemia (17%), dimorphic anemia (10%) and macrocytic anemia (2.7%). (Table 7)

Table 7. Comparative study of morphological patterns in pediatric anemias

Morphological type	Present study	Kapur et al
Microcytic hypochromic	**62.8%**	**43.2%**
Normocytic normochromic	**5.7%**	**17%**
Normocytic hypochromic	**2.9%**	**27%**
Dimorphic	**17.1%**	**10%**
Hemolytic	**11.4%**	**0%**
Macrocytic	**0%**	**2.7%**

CONCLUSION

Determination of morphological patterns of pediatric anemias by baseline hematological investigations helps in determining the probable etiological factor. This contributes in effective patient management without having to submit the patient to time- consuming and expensive advanced investigations. It is especially suitable for a rural tertiary care hospital where appropriate and cost effective treatment is required and most appreciated.

Our study indicates that iron deficiency is the underlying etiologic factor in microcytic hypochromic patterns. Serum iron assay is not mandatory if it is unaffordable to the patient. The clinician may initiate iron therapy in all such cases with microcytic hypochromic pattern of anemia. Dimorphic blood picture may be associated with combined iron and Vitamin B12/Folate deficiencies. In an ideal situation, therapy should be initiated after serum assays or bone marrow examination. But in an impossible situation, the clinician may resort to a combination therapy. But in cases of hemolytic anemias, the morphologic hemolytic pattern alone does not suffice to initiate treatment and serum electrophoresis becomes mandatory for confirmation of etiologic factor in such cases.

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CONFLICT OF INTEREST

None declared**.**

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