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Original Research Paper

Hematology

A CLINICO-HEMATOLOGICAL PROFILE OF MICROCYTIC HYPOCHROMIC ANEMIA IN CHILDREN: AN OBSERVATIONAL STUDY

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ABSTRACT Microcytic hypochromic anemia is anemia classification based on the morphology of anemia . The causes of microcytic hypochromic anemia may be either due to iron deficiency anemia, anemia of inflammation, or thalassemia. . To investigate the causes of Microcytic Hypochromic anaemia at a tertiary care centre in NALGONDA. After obtaining valid written consent, cases of microcytic hypochromic anaemia were selected from the OPD . The whole haematological and biochemical investigations were sent for anaemia workup. The study comprised 44 patients with microcytic hypochromic disorder. The study comprised subjects ranging in age from 1 TO 11 years. 42% were men and 58% were women. Anemia is not an illness in and of itself, but rather a symptom of another, hence finding the underlying cause is significantly more important. The diagnosis of microcytic hypochromic anaemia is insufficient in the absence of an underlying cause. Special precautions will be made to determine the cause of iron deficient anaemia. The thalassemia trait must also be diagnosed in order to minimise excessive iron supplementation and for family screening.

KEYWORDS : Iron-Deficiency Anemia, Anemia of Chronic Disease, Microcytic Hypochromic Anemia

INTRODUCTION

Anaemia, whether clinically overt or not is a common condition encountered by the family physician. The most commonly encountered disorders manifesting with mild microcytic hypochromic anaemia are iron deficiency anemia (IDA) and Thalassemia Trait (TT)[1,2]. Hypochromic microcytic aneamia could also be due to anaemia of chronic disease or lead poisoning. The establishment of an accurate diagnosis is of great importance in ensuring correct treatment. Administration of iron to a patient with hypochromic aneamia due to a cause other than iron deficiency is not only useless but also leads to undesirable effects of increase in body iron stores. Thalassemia Minor and Thalassemia Intermedia may pass off as iron deficiency anaemia, if only peripheral smear was the sole diagnostic modality.

β-Thalassemia is also an iron loading anemia, meaning that thalassemic patients have a dramatic increase in iron absorption from the gut due to their increased erythropoietic rate. Together with the iron influx from chronic transfusions the setting of iron overload is observed in thalassemic patients. Inadvertent iron therapy will prove detrimental in such situations[3].

Iron plays an essential role in many important biochemical processes . As with all nutrients, the requirement for iron is greater during periods of rapid growth and differentiation such as in the late fetal and neonatal period. 2 Consequently, poor iron homeostasis during this period can result in disordered development. Inadequate tissue iron levels can lead to reduced erythropoiesis and poor O2-carrying capacity. The nervous system, which develops rapidly during the late fetal and early neonatal period, seems to be particularly susceptible to iron deficiency and excess[4] . Also, Iron excess can have severe effects on neuron development . Thus, events occurring in early life can have long-lasting effects on neuronal function in the adult. Excessive iron in the circulation leads to abnormal accumulation in organs such as liver, spleen and heart, leading ultimately to liver disease, cardiac dysfunction, arthropathy, gonadal insufficiency and other endocrine disorder[5]

MATERIAL AND METHODS

Study Design:- Prospective study

Study Population:- Patients aged 1-12 years with microcytic hypochromic anaemia.

Sample Size:-

A total of 44 patients with hypochromic microcytic anaemia were subjected to assessment of hematological profile. Approximately 500 samples of hypochromic microcytic anaemia are received in the department of pathology per year representing 3.67% of incidence.

Study Period:-

One and a half years [February 2022 to August 2023].

METHODOLOGY:-

Patients aged 1-12 years with microcytic hypochromic anaemia are selected with following inclusion and exclusion criteria.

Inclusion Criteria:-

Age group 1-12years. Patients with clinical symptoms of anaemia. Haemoglobin level:AGE 1-6 < 10.5g/dl , Age 7-12 < 11g/dl

Exclusion Criteria:-

Age below 1year and above 12 years. Peripheral smear with dimorphic picture. H/O transfusion within past two months.

Detection Principle:

This instrument performs blood cell count by DC detection method. Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into the each transducer. The transducer chamber has a minute hole called aperture. On both sides of the aperture there are electrodes between which flows the direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As the current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell size is plotted by determining the pulse size. Also analyzing a histogram makes it possible to obtain various analysis data.

To analyze the haemoglobin by automated methods, the cyanmethaemoglobin or oxyhaemoglobin methods have so far been the mainstream.

Serum Iron Level Assay:

Method: Ferrozine method without deproteinization.

Serum Iron Binding Capacity:
Method: Spectrophotometric assay.

Ferritin Estimation:
Method: Fully automated biodirectionally interfected chemiluminescent immuno assay.

OBSERVATION AND RESULTS

Newly diagnosed cases of microcytic hypochromic anaemia admitted to nalgonda Medical College hospital with no prior blood transfusion or iron treatment were randomly enrolled. A total of 44 cases were enrolled as per inclusion and exclusion criteria.

AGE:
Patient with Microcytic Hypochromic Anaemia with age between 1-12 years were included in this study. Out of 44 cases, 27.3% were below 4 years, 36.4% were between 5-8 years & 36.4% were between 8-12 years.

Table-1: Age Incidence

Age (in years)	Study population	Percent
1-4	12	27.3
5-8	16	36.4
9-12	16	36.4
Total	44	100.0

[n=Total number of cases (44)]

SEX
Among the randomly enrolled 44 patients, 25 were girls while the remaining 19 were boys. Girls constituted 56.82%, boys constituted 43.18%.

Severity Of Anaemia
Mild anaemia (Hb 10-12 g/dl) was present in 26 cases, moderate anaemia (Hb 8-9.9 g/dl) in 13 cases and severe anaemia (Hb <8 g/dl) in 5 cases.

Table-2: Severity Of Anaemia:

Hb (g/dl)	Study population	Percent
< 8	26	59.1
8 – 9.9	13	29.5
10 – 11	5	11.4
Total	44	100.0

Mean Corpuscular Volume (MCV)
97.7% of study population had MCV below 80 fl.

Table-3: MCV Distribution

MCV(fl)	Study population	Percent
<80	43	97.7
80 – 100	1	2.3
Total	44	100.0

MEAN CORPUSCULAR HAEMOGLOBIN
93.2% had MCH below 25pg.

Table-4: MCH Distribution

MCH (pg)	Study population	Percent
25 & below	41	93.2
26 – 34	3	6.8
Total	44	100.0

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION
95.5% had MCHC below 30 g/dl.

Table-5: MCHC Distribution

MCHC (g/dl)	Study population	Percent
30 & below	42	95.5
31 – 37	2	4.5
Total	44	100.0

Serum Ferritin:
Out of 44 cases, 52.3% had S. Ferritin below 12ng/dl, 20.5 % had S. Ferritin between 12-50ng/dl & 27.3% had S. Ferritin above 50ng/dl.

Table-6: Serum Ferritin

S. Ferritin (ng/dl)	Study population	Percent
<12	23	52.3
12-50	9	20.5
>50	12	27.3
Total	44	100.0

Age Wise Statistical Analysis
Mean haemoglobin level does not vary significantly with age. When ANOVA test was conducted (F=2.765; P>0.05) the mean haemoglobin was highest in the age group of 9- 12 years (7.76 g/dl) and lowest in the age group of 1-4 years (5.93 g/dl).]

Table-7: Haemoglobin In Various Age Groups

Age (in years)	Haemoglobin (g/dl) Mean	S.D	Study population
1 – 4	5.93	2.08	12
5 – 8	6.33	2.13	16
9 – 12	7.76	2.39	16
Total	6.74	2.31	44

RBC Count In Various Age Groups
Mean RBC counts varied significantly with age. When ANOVA test was conducted (F= 4.799; P<0.05*) the mean RBC count was highest in the age group of 9- 12 years (3.81million/ mm^3) and lowest in the age group of 5-8 years (2.73 million/mm^3). (*- significant at 5% level)

Table 8: RBC Count In Various Age Groups

Age (in years)	RBC count (million/mm^3) Mean	S.D	Study population
1-4	2.82	0.81	12
5-8	2.73	0.96	16
9-12	3.81	1.31	16
Total	3.15	1.16	44

[SD= Standard deviation**]**

HAEMATOCRIT IN VARIOUS AGE GROUPS

Mean haematocrit does not vary significantly based on age. When ANOVA test was conducted (F= 3.098 ; P>0.05) the mean haematocrit was highest in the age group of 9- 12 years (22.33%) and lowest in the age group of 5- 8 years (17.10%).

Table-9: Haematocrit (HCT) In Various Age Groups

Age (in years)	HCT (%) Mean	S.D	Study population
1 – 4	17.59	6.96	12
5 – 8	17.1	6.3	16
9 – 12	22.33	6.2	16
Total	19.13	6.76	44

DISCUSSION

The current study mainly focused on the utility of serum iron profile, hemoglobin electrophoresis and peripheral smear in microcytic hypochromic anaemia. This study also attempted to elucidate the diagnostic accuracy of seven indices to discriminate mild to moderate Iron deficiency from β - Thalassemia.

AGE, SEX INCIDENCE & SEVERITY OF ANAEMIA:
The present study included patients between the age group of 1- 12 years. Two thirds of anaemic children were between the age of 5 & 12 years. This could be attributed to the increasing nutritional demands of growth spurt and puberty compounded by less attention to nutrition, amidst demanding academic pressures.

In the present study, girls with microcytic hypochromic

anaemia out number the boys. This female preponderance could be due to less care of the girl child in Indian settings. Distribution of Hb, RBC, HCT, MCV, MCH, MCHC, RDW do not vary significantly in both sexes.

Mild anaemia (Hb10- 12 g /dl) was present in 26 cases (11.4%), moderate anaemia (Hb 8 -9.9g/dl) in 13 cases (29.5%) and severe anaemia (Hb <8 g/dl) in 5 cases (59.1%).

Prevalence Of IDA:

A study conducted by **Mohemmed et al.** in the 2006 **& Looker et al.** in 1997, found that IDA is most prevalent in children.

This finding correlates with the present study where 72.73% of microcytic hypocromic anaemia was found to be due to Iron deficiency.

[β-Thalassemia trait; 6.82%, β-Thalassemia major; 6.882% & anaemia of chronic disease; 13.64%]..

Value Of Red Cell Indices In Differentiating Between BETA-TT & IDA:

Mean haemoglobin varied significantly between IDA, β-TT. The mean Hemoglobin in IDA patient was 8.99g/dL whereas for β-TT 10.57g/dL. Mean MCV varied significantly between IDA, β-TT, mean MCV in IDA patient 68.45 (fl) whereas for β-TT 38.91 (fl).

MCV is known to be significantly low in β-Thalassemia as compared to Iron deficiency anaemia. Mean MCH does not vary significantly between IDA, β-TT, mean MCH in IDA patient was 20.79 pg whereas for β-TT 17.04 pg. Mean MCHC does not vary significantly between IDA, β-TT, mean MCHC in IDA patient was 23.83g/dl whereas for β-TT 27.52 g/dl.

RED CELL INDICES AND ITS USES:

A study conducted by **M.A.Ehani et al.** included 284 patients aged (range 10 – 38 years), this study utilized 4 indices including England and Fraser Index , Mentzer Index, Srivastava index & RBC count to discriminate 130 cases of IDA & 154 cases of b TT. Youden's index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity , and was first used by **Demir et al.** Youdens index was calculated, showed MI (90.1) to be superior to Srivastava index (74.2) and England & fraser index(68.7) in that order.4

Table-41: Following Table Shows Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value Found In The Present Study:

Indices	Anaemia	Sensitivity	Specificity	PPV	NPV	Y.I
Mentzer index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
Shine and Lal Index	IDA	9.1	100.0	100.0	23.1	9.1
	B-TT	100.0	9.1	23.1	100.0	
England Fraser Index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
Srivasthava index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
Green & king index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
RBC Distribution width index	IDA	100.0	33.3	84.6	100.0	33.3
	B-TT	33.3	100.0	100.0	84.6	
Ricerca index	IDA	100.0	0.0	78.6	0.0	0.0
	B-TT	0.0	100.0	0.0	78.6	

The present study found that, to differentiate mild to moderate IDA (Hb 8.5 – 11 g/dl) from β-TT Mentzer index, England and Fraser index, Srivastava index & Green and king index had highest specificity as well as Youden's index. However RBC distribution width index was found to have reasonable specificity and sensitivity when compared to Shine and lal index & Ricerca index.

Table- 42: Hematological Parameter

Parameter	Ehana et al.		Present study	
	IDA	B-TT	IDA	b-TT
Hb (g/dl)	9.3(+/-) 1.89	11.24(+/-) 1.37	8.99(+/-)0.81	10.57(+/-) 0.23
MCV (fl)	70.04(+/-) 7.94	62.02 (+/-) 4.57	68.45(+/-) 9.20	38.91(+/-) 0.16
MCH (pg/cell)	21.30 (+/-) 3.52	19.68 (+/-) 1.53	20.79(+/-) 3.24	17.04(+/-) 0.8
MCHC (g/dl)	29.88(+/-) 2.86	30.93(+/-) 1.71	23.83(+/-) 4.49	27.52(+/-) o.54

A study conducted by **Ehana et al.**[38] found that MCHC was low in Iron deficiency anaemia, whereas MCV, MCH were low in Thalassemia & MCHC was normal. The present study showed similar results.

SUMMARY AND CONCLUSION

- In the present study of 44 children with microcytic hypochromic anaemia 12 were in 1-4 year age group (27.3%), 16 were in 5-8 year age group (36.4%) & 16 were in 9-12year age group (36.4 %)
- Out of 44 patients studied, 56.8% were girls, 43.2% were boys.
- Mild anaemia was observed in 11.4% of children; moderate anaemia was observed in 29.5 % of children, severe anaemia was observed in 59.1% of cases
- Out of 44 cases, IDA constituted 72.73%, β-Thalassemia trait constituted 6.82%, β-Thalassemia major constituted 6.882% & Anaemia of chronic disease constituted 13.64%.
- Following median values were obtained:

For IDA- RBC count- 2.58million/mm^3, Hemoglobin- 5.85g/dl, MCH- 19.2 pg, MCHC- 22.45g/dl, RDW- 50.4 fl, S.Iron- 22μg/dl, Serum Ferritin- 4.67ng/dl & TIBC- 411.35μg/dl.

- β Thalassemia trait: -

RBC count 5.36 million/mm^3, MCV-62.1fl, MCH- 20.6 pg, MCHC-22.1g/dl, S.Iron-199.8μg/dl, Serum Ferritin-307.3ng/dl & TIBC- 267.1μg/dl.

Mean haemoglobin, MCV, MCH, RDW were lower in boys. Mean RBC, MCHC, HCT were higher in girls. Mean Serum Iron & Ferritin were low and TIBC was high in girls.

Out of 44 cases, 14 with mild to moderate anaemia caused clinical confusion between Iron deficiency anaemia and β-Thalassemia trait. After doing Iron profiles and electrophoresis 11 were concluded as Iron deficiency anaemia, and 3 were concluded as β-Thalassemia trait.

In remaining 30 cases, 3 turned out to β-Thalassemia major and 6 were found to be anaemia of chronic disease.

In 6 children with anaemia of chronic disease, 3 were suffering from pneumonia, other two were suffering from urinary tract infection and remaining one child was suffering from tuberculosis.

MCV was found to be lower in β-Thalassemia Trait than in IDA, which could have a useful application in differentiating these two conditions.

7 indices were used to differentiate mild to moderate Iron deficiency anaemia and β-Thalassemia trait. For differentiating these two entities, Youdens index showed that Mentzer index, England and Fraser index, Srivastava index & Green and King index were equally superior to RBC Distribution width index, Shine and lal index & Ricerca index

in that order[6-7].

In microcytic hypochromic anaemia peripheral smear, red cell indices, Serum Iron profile including Serum Iron, TIBC, Serum Ferritin and Haemoglobin electrophoresis were found to be useful parameters in the precise assessment of anaemia and its type[8-9].

Hb Electrophoresis, Serum Iron profile & Red cell indices are complementary to each other in the precise diagnosis of microcytic hypochromic anaemia of varied etiology, which would enable comprehensive wholesome treatment[10].

REFERENCES

1. Wharton BA. Iron deficiency in children: detection and prevention. Br J Haematol 1999; 106: 270 – 80.
2. Lukens JN. The thalassemias and related disorders: an overview. In: Lee GR, et al., editors. Wintrobe's Clinical Hematology. 10th ed. Giza: Mass Publishing, 1999: 405-33.
3. Adamsky K, Weizer O, Amariglio N, et al. Decreased hepcidin mRNA expression in thalassemic mice. *British Journal of Haematology*. 2004;124(1):123–124.
4. Breda L, Gardenghi S, Guy E, et al. Exploring the role of hepcidin, an antimicrobial and iron regulatory peptide, in increased iron absorption in β-thalassemia. *Annals of the New York Academy of Sciences*. 2005;1054: 417–422.
5. Gardenghi S, Marongiu MF, Ramos P, et al. Ineffective erythropoiesis in Zβ-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood. 2007; 109(11):5027–5035.
6. Kattamis A, Papassotiriou I, Palaiologou D, et al. The effects of erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major.B *Haematologica*. 2006; 91(6):809–812.
7. Dunn LL, Rahamanto YS, Richardson DR. Iron uptake andmetabolism in the new millennium. *Trends Cell Biol*. 2007; 17(2):93–100.
8. Fredriksson A, Schroder N, Eriksson P, Izquierdo I, Archer T. Maze learning and motor activity deficits in adult mice induced by iron exposure during a critical postnatal period. *Brain Res Dev Brain Res*. 2000;119(1):65–74.
9. Pin˜ ero DJ, Li NQ, Connor JR, Beard JL. Variations in dietary.
10. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long lasting neural and behavioural effect of iron deficiency in infancy. *Nutr Rev*. 2006;64(5 pt 2):S34–S41.