DETECTION OF HAEMOGLOBINOPATHIES USING HAEMOGLOBIN ELECTROPHORESIS IN MICROCYTIC HYPOCHROMIC ANAEMIA IN PAEDIATRIC POPULATION OF SOUTHERN ODISHA

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ABSTRACT

BACKGROUND

Anaemia is the most common symptom in the developing countries including India where under nutrition is prevalent besides haemoglobinopathy is also prevalent in this geographic region. Haemoglobinopathies especially thalassaemia minor present in the paediatric age group with microcytic hypochromic anaemia and hence often misdiagnosed and treated wrongly as iron deficiency anaemia. Simpler cost-effective techniques like study of haematological indices by performing Complete Blood Count (CBC) and in suspicious cases haemoglobin electrophoresis the exact diagnosis can be established at the earliest.

MATERIALS AND METHODS

Under aseptic condition, 5 mL of blood collected in K_2EDTA Vacutainer. One part was analysed in cell count analyser. Other part was used to prepare hemolysate and subsequently gel electrophoresis.

RESULTS

Gel Electrophoresis is done in 210 cases of microcytic hypochromic anaemia which is detected by haemogram assay, 21% of cases were diagnosed to be thalassaemia and 79% were iron deficiency anaemia.

CONCLUSION

Iron-Deficiency Anaemia (IDA) is the commonest cause of microcytic hypochromic anaemia, the second being thalassaemia. Differential diagnosis is based on complete haemogram and peripheral smear. Serum iron profile study and haemoglobin electrophoresis are must for confirmation of diagnosis.

KEYWORDS

Haemoglobinopathies, Haemoglobin Electrophoresis, Microcytic Hypochromic Anaemia, Paediatric Population.

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BACKGROUND

Anaemia is a global health problem more prevalent in the developing countries like India. The National Family Health Survey (NFHS) III data showed the prevalence of anaemia among paediatric age group less than five years of age to be around 70%.[1] The prevalence rates of anaemia in the children of 6 to 59 months age group in different states of anaemia is given in the table below. Although, some studies have suggested a decline in prevalence of anaemia,[2] the most recent reports showed an increase in its frequency among low income group and in underdeveloped countries.[3] The complications of anaemia are well known, but to mention a few in children are poor mental performance and behavioural abnormalities.[4]

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	Mild Anaemia	Moderate Anaemia	Severe Anaemia	Total Anaemia
India	26.3	40.2	2.9	69.5
North				
Delhi	26.3	30.0	0.7	57.0
Haryana	25.8	42.2	4.3	72.3
Himachal Pradesh	25.7	26.8	2.2	54.7
J and K	25.8	30.4	2.4	58.6
Punjab	21.7	38.1	6.6	66.4
Rajasthan	22.8	40.2	6.7	69.7
Uttaranchal	28.5	30.6	2.3	61.4
Central				
Chhattisgarh	24.0	45.2	2.0	71.2
Madhya Pradesh	27.1	43.6	3.4	74.1
Uttar Pradesh	25.4	45.0	3.6	73.9
East				
Bihar	29.6	46.8	1.6	78.0
Jharkhand	29.3	39.1	1.9	70.3
Orissa	28.9	34.7	1.6	65.0
West Bengal	30.0	29.4	1.5	61.0

Table 1. Prevalence Rate of Anaemia in the State of Study, i.e. Odisha is High (65%)

States	N	Normal (>11 g/dL)	Anaemia			
			Mild (10-11 g/dL)	Moderate (7-10 g/dL)	Severe (<7g/dL)	Total (CI)
Kerala	369	66.3	20.1	13.3	0.3	33.7 (28.8,38.4)
Tamilnadu	407	37.3	22.4	36.1	4.2	62.7 (58.0,67.3)
Karnataka	425	33.6	20.7	43.3	2.4	66.4 (61.9,70.9)
Andhra Pradesh	448	29.2	24.8	42.2	3.8	70.8 (66.6,75.0)
Maharashtra	404	40.9	20.5	35.6	3.0	59.1 (54.3,63.9)
Madhya Pradesh	394	35.3	24.1	38.1	2.5	64.7 (60.0,69.4)
Orissa	407	7.6	22.1	69.8	0.5	92.4 (89.8,95.0)
West Bengal	437	18.8	34.1	47.1	0.0	81.2 (77.5,84.9)
Pooled	3291	33.1	23.7	41.1	2.1	66.9 (65.3,68.5)

Table 2. Prevalence (%) of Anaemia among Children of Age One to Less than Five Years

Table 2 shows comparative levels of anaemia in children among the different states of India. Odisha have anaemia prevalence of more than 70%.[5,6]

Microcytic anaemias are among the most common types of anaemia encountered by physicians in general hospitals and outpatient clinics.[7] The differential diagnoses include Iron-Deficiency Anaemia (IDA), thalassaemias and other variant haemoglobins such as HbS, HbE and Hb Lepore, anaemia of inflammation, congenital sideroblastic anaemia and lead poisoning.[4,8] Among these, the two most common causes are iron deficiency and thalassaemia.

Global prevalence of iron deficiency in young children is 43%,[9] but children between 1-2 years are at greatest risk and the major factors in this age group is due to the demand and supply gap resulting from prolonged consumption of cow's milk, delayed weaning, poor dietary habits (use of foods with low bioavailability of iron), worm infestations, etc.

Haemoglobinopathies are the most common monogenic disorders of erythrocytes. Ten percent of the total world thalassaemics are born in India every year. It has been estimated that the prevalence of pathological haemoglobinopathies in India is 1.2/1,000 live births and with approximately 27 million births per year. Within this, overall disease classification, a 1989 WHO Working Group on guidelines for the control of haemoglobin disorders estimated a 3.9% carrier frequency for β-thalassaemia in India encompassing all types of β-thalassaemia trait. This health burden emphasises the need for prenatal diagnosis and carrier status detection to contain the disease and reduce the load of the mutant alleles in the gene pool.[10]

An accurate diagnosis of β-thalassaemia carriers, homozygous patients and identification of different structural haemoglobin variants is important for epidemiological studies as well as for management and prevention of the major haemoglobin disorders. Differentiating mild or moderate IDA from thalassaemia trait can be a diagnostic dilemma as both conditions share many characteristics. Obviously, a correct diagnosis in patients with microcytic anaemia is important. It can provide an indication for supplementing iron to IDA patients for avoiding unnecessary iron overload in thalassaemia traits and of course also for preventing severe and lethal forms of thalassaemia syndromes.

My research provides glimpses of microcytic hypochromic anaemia especially IDA and thalassaemia with special emphasis on early diagnosis of thalassaemias.

AIMS AND OBJECTIVES

1. To study the role of various haematological indices and peripheral smear examination findings in evaluating microcytic hypochromic anaemias.
2. To evaluate the role of haemoglobin electrophoresis in the diagnosis of haemoglobinopathies in cases of microcytic hypochromic anaemia.

MATERIALS AND METHODS

A 2 years prospective cross-sectional study with 210 patients of paediatric age group was undertaken in the Department of Pathology, MKCG Medical College and Hospital, Berhampur, during the period October 2013 to October 2015 in which all paediatric patients who presented with pallor and were detected to have microcytic hypochromic anaemia on peripheral examination were included in the study group and anaemias other than microcytic hypochromic anaemia are excluded.

Study Protocol

Written informed consent, detailed clinical history including family history with special emphasis on consanguinity of marriage and transfusion history was obtained from the patient. 3 mL of EDTA blood was collected in a Vacutainer. All samples were subjected for Complete Blood Count (CBC) by the fully automated 5 part cell counter Sysmex XT-2000i and in each case a thin blood smear was prepared and stained with Leishman stain.

The stained slides were evaluated under microscope to confirm microcytic hypochromic morphology and to exclude any other pathology. All cases of microcytic hypochromic anaemia, CBC and PB were subjected for iron profile study- Sr. iron, TIBC and % saturation to confirm iron deficiency aetiology.

All non-iron deficiency microcytic hypochromic anaemia samples were subjected for electrophoresis at alkaline pH and later for HPLC for confirmation.

Statistical Analysis

Student's t-test and analysis of variance was used for comparing the means. All results were at a preset significance level of p=0.05 and with 95% confidence intervals.

It was done using SPSS ver. 21.

OBSERVATION

Two years prospective cross-sectional study with 210 patients of paediatric age group was undertaken to study the role of electrophoresis in diagnosing the microcytic hypochromic anaemia cases.

Study Groups	Number of Patients	%
Iron Deficiency Anaemia	166	79.05
Thalassaemia	44	20.95
Total	**210**	**100.0**

Table 3. Distribution of Cases in Two Study Groups in the Present Study

A total 210 microcytic hypochromic anaemia cases were included in the study. Iron profile study confirmed 166 cases (79.05%) as IDA and rest 44 cases (20.95%) were confirmed to be thalassaemia.

Age in Years	Iron Deficiency Anaemia		Thalassaemia	
	Number	%	Number	%
<1 year	8	4.82	4	9.09
1-5 years	65	39.15	23	52.27
6-10 years	69	41.56	15	34.09
11-14 years	24	14.45	2	4.5
Total	166	100.0	44	100.0
Inference	**No statistically significant association of age group was observed with either IDA or thalassaemia (p=0.412)**			

Table 4. Age Distribution of Iron Deficiency Anaemia and Thalassaemia

Table 4 shows that majority of patients both in IDA and thalassaemia were in the 1-5 years and 6-10 years age group accounting for 80.71% and 86.36%, respectively.

Family History	Number (n=44)	%
Present	19	43.18
Not present	25	56.82

Table 5. Family History of Consanguineous Marriage in Cases of Thalassaemia (n=44)

19 out of 44 patients, i.e. 43.18% of thalassaemics gave a positive history of consanguinity of marriage.

		Number (n=44)	%			Number (n=44)	%			Number (n=44)	%
Clinical Symptoms	Breathlessness	44	100.0	**Signs**	Pallor	44	100.0	**Complications**	Growth impairment	29	65.9
	Gen weakness	44	100.0		Hepatosplenomegaly	44	100.0		Iron overload	27	61.36
	Mass per abdomen	44	100.0		Frontoparietal bossing	29	65.9		Cardiac failure	1	2.27
	Jaundice	6	13.63		Jaundice	6	13.63				

Table 6. Study of Clinical Spectrum (Symptoms + Signs + Complications)

As evident from Table 6, all thalassaemics presented with breathlessness, generalised weakness and mass per abdomen.

	Hb. Concentration (g/dL)		MCV (fl)		RDW (%)		Total Platelet Count in lakhs/cumm		Total WBC Count	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All Cases	6.11	2.15	64.97	13.43	17.21	3.32	3.19	1.47	9528.914	50.919
IDA	6.31	2.23	68.72	11.16	18.27	2.83	3.31	1.54	9413.855	49.547
Thalassaemia	5.35	1.62	50.81	11.80	13.06	1.11	2.73	1.06	9963.000	56.132
p value	0.008		0.001		0.001		0.052		0.853	

Table 7. Laboratory Investigations - Haemogram

		IDA	Thal. Major	Thal. Intermedia	Thal. Minor
Hb conc.	Mean±SD	6.31±2.23	5.1±1.6	6.8±1.3	8.02±0.96
TRBC	Mean±SD	3.51±1.09	2.71±1.18	3.52±1.21	4.52±1.25
	p value	0.001	0.001	0.001	0.014
MCV	Mean±SD	68.72±11.15	48.61±11.39	60.00±12.22	64.63±10.84
	p value	0.046	0.046	1.0	1.0
MCH	Mean±SD	18.42±4.34	15.61±5.55	16.00±5.14	17.41±4.37
	p value	1.0	1.0	1.0	1.0
MCHC	Mean±SD	26.95±7.55	23.45±6.09	24.68±4.54	25.95±6.14
	p value	0.001	0.001	0.001	0.001
RDW	Mean±SD	18.31±2.80	12.95±1.09	13.35±0.79	13.94±1.55
	p value	0.001	0.001	0.001	0.001

Table 8. RBC Indices

Both table 7 and 8 depicts the degree of anaemia decreases in severity from thalassaemia major to minor. In comparison to IDA, thalassaemia minor cases are less anaemic. The MCV variation was statistically significant between thalassaemia and IDA. MCV values did not vary significantly in different thalassaemia syndromes. RDW values did not vary significantly in different thalassaemia syndromes. But, it showed a statistically significant lower value in all thalassaemics compared to IDA patients. The average PCV value was significantly lower in thalassaemia in comparison to IDA. The average TRBC count in thalassaemia major was lower than IDA. However, erythrocytosis was observed in both thalassaemia intermedia and thalassaemia minor when compared with IDA.

	Serum Iron in µg/dL		Total Iron Binding Capacity (µg/dL)		Transferrin Saturation Value in Percentage	
	Mean	SD	Mean	SD	Mean	SD
All cases	77.04	64.94	379.09	91.19	23.84	27.37
IDA	46.40	12.96	416.95	51.77	11.55	4.57
Thalassaemia	192.66	50.52	226.67	37.28	73.98	17.49
p value	0.001		0.001		0.001	

Table 9. Iron Profile Study

Table 9- IDA cases showed a low serum iron value and the thalassaemics showed iron overload. IDA cases showed a high TIBC value and the thalassaemics showed a lower TIBC.

It shows a statistically significantly low transferrin saturation value in cases of IDA whereas the same in thalassaemia cases is 73.98±17.49.

	Haemoglobin Electrophoresis in Thalassaemia Cases		HPLC in Thalassaemia	
Haemoglobinopathies	Number of Cases (n=44)	Percentage	Number of Cases (n=44)	Percentage
β-thalassaemia major	21	47.7	24	54.6
β-thalassaemia minor	10	22.8	09	20.5
β-thalassaemia intermedia	06	13.7	06	13.7
Sβ-thalassaemia	03	6.8	03	6.8
δβ-thalassaemia	03	6.8	-	-
Eβ-thalassaemia	01	2.2	01	2.2
E-heterozygous	-	-	01	2.2

Table 10. Haemoglobin Electrophoresis in Thalassaemia Cases

Table 10- Haemoglobin electrophoresis in thalassaemia cases showed out of 44 (100%) cases, maximum number 21 of patients were having β-thalassaemia major (47.7%), followed by (in the descending order of incidence) β-thalassaemia minor (10, 22.8%), β-thalassaemia intermedia (6, 13.7%), Sβ-thalassaemia (3, 6.8%), δβ-thalassaemia (3, 6.8%), Eβ-thalassaemia (1, 2.2%) cases.

HPLC in thalassaemia cases showed out of 44 (100%) cases maximum number of patients were having β-thalassaemia major followed by (in the descending order of incidence) β-thalassaemia minor, β-thalassaemia intermedia, Sβ-thalassaemia, Eβ-thalassaemia and E-heterozygous. As evident from the above table, the results of electrophoresis and HPLC are comparable.

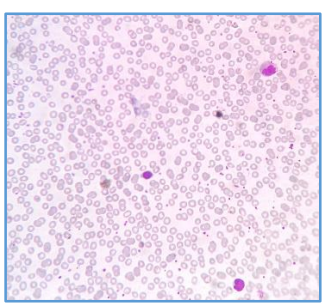

Figure 1. 40x showing PS of microcytic hypochromic RBCs with mild anisocytosis - Thalassemia minor

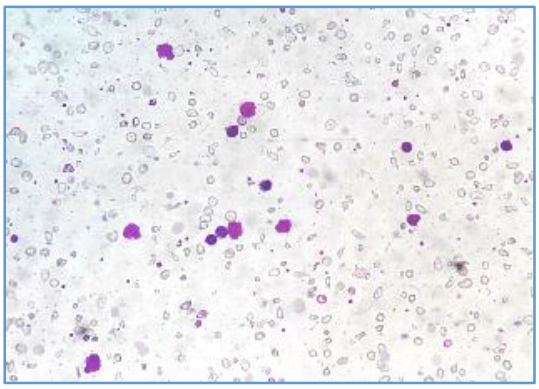

Figure 2. 40x Showing PS of Microcytic Hypochromic RBCs with Good Number of Normoblasts and Target Cells – Thalassemia Major

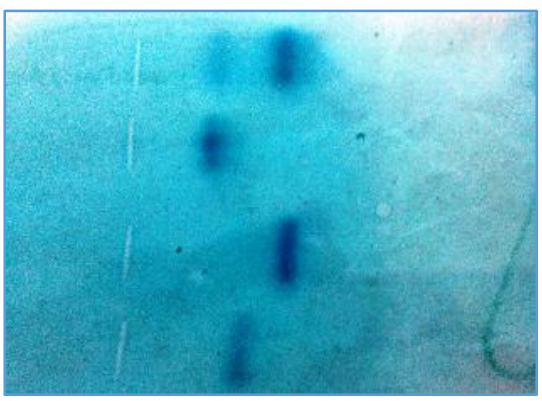

Figure 3. Showing Electrophoretic Pattern of Thalassemia Major

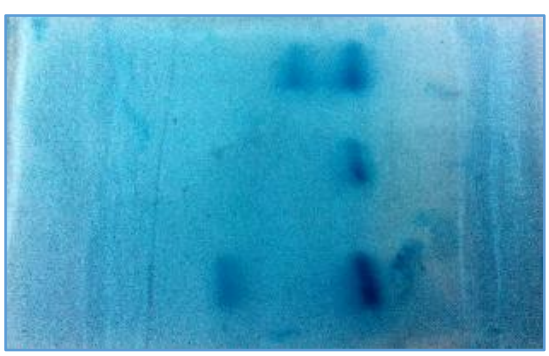

Figure 4. Showing Electrophoretic Pattern of Eβ Thalassemia

Figure 5. Showing Automated Hematology Analyzer (Sysmex XT-2000i)

Figure 6. Electrophoresis Setup

DISCUSSION

The present study is a small effort to put together on important aspects of anaemia, i.e. its cause, presentation and early diagnosis using an automated cell counter and electrophoresis.

The prevalence of IDA is more compared to thalassaemias in our study. Most of the studies agree to the finding, but the rates vary. Mach-Pascual S, Darbellay R, Pilotto P A, Beris P in 1996[11] studied 466 patients with Microcytic Hypochromic Anaemia (MCV <82 fL) and found that IDA was the first cause of microcytosis (35.2%), α-thalassaemia (31.1%) and β-thalassaemia heterozygous state (18.9%). Ahmed et al in 1996[12] observed the prevalence of β-thalassaemia and IDA to be 6% and 43%, respectively. The variation can be due to the geographic and socioeconomic variations of the study group and the sample size.

In my study, observing the bands on the gel slab and correlating with the clinical and laboratory parameters, the inferences made from haemoglobin electrophoresis are maximum number patients as β-thalassaemia major (47.7%), followed by β-thalassaemia minor, β-thalassaemia intermedia,[3] Sβ-thalassaemia, δβ-thalassaemia and Eβ-thalassaemia. But, to quantitate the different Hb variants, HPLC was done and the results of haemoglobin electrophoresis is comparable with that of HPLC as was the observations in the studies of Shafi Mohammad Khosa, Muhammad Usman, Moinuddin Moinuddin, Hassan Osman Mehmood and Khansa Qamar in 2015.[13] With the help of electrophoresis, all cases of β-thalassaemia major were picked up correctly, but some thalassaemia traits and heterozygous cases could not be diagnosed for which quantification of Hb variants is a must. In 2002, ICSH[14] also recommends that β-thalassaemia minor should be diagnosed by determining HbA_2 levels using techniques such as cellulose acetate Hb electrophoresis or HPLC.

The increased prevalence of β-thalassaemia was also observed in the study of Mohan N, Sarkar R in 1994[15] whereas in the 466 cases the said prevalence was 80% in Asian Indians. Study of Kishore B, Khare P, Gupta RJ, Bisht S, Majumdar K in 2007[16] on HbE disease in Indian population have also noted a similar prevalence of Eβ-thalassaemia. The observed prevalence of haemoglobinopathies in the

study of Jignasa N Bhalodia et al in 2015[17] was very low. The study detected only 43 cases out of 500 anaemia cases to have abnormal haemoglobin fractions on HPLC of which 26 cases (5.2%) were β-thalassaemia trait, the predominant abnormality. Among the rest 6 (1.2%) were of sickle cell trait, 4 (0.8%) were high HbF patients and 2 (0.4%) were HbD Punjab heterozygous patients.

As per age is considered, according to Astaldi, Tolentino and Sacchetti in 1952[18] several grades of the disease were recognised. A severe form of anaemia manifesting in early infancy and often results in death in the first year. The second type is slightly less severe form of the disease, which usually first manifest in second half of first year and in this form the child often survives until school age and a still milder form, which is usually diagnosed in second year of life is compatible with survival up to adult age. The cases those were diagnosed within six months of life represented the severe form, i.e. the β-thalassaemia major and cases diagnosed after six months represented less severe forms. In our study, only 4 cases were diagnosed before completion of one year.

Mohan N, Sarkar R in 1994[15] studied the haematological status of β-thalassaemia in the ethnic groups particularly from Southern India and observed a high degree of consanguinity of marriage, which correlates well to our study.

The study of spectrum of clinical signs and symptoms also matches with the observations made by Swaroop Mitra et al in 1983[19] and also Tyagi S et al in 2003.[20]

The iron profiles of IDA in the present study correlated well with that of Bainton and Clement A Finch in 1964[21] as evident from the table below-

	Serum Iron	TIBC	% Saturation
Bainton-Finch study (1964)	10-61 µg/dL (mean=28 µg/dL)	170-460 µg/dL (mean=346 µg/dL)	2-16% (mean=7%)
Present study	18-69 µg/dL (mean 46.08±12.92 µg/dL)	300-512 µg/dL (mean 416.95±51.77 µg/dL)	3.16-21.2% (mean 10.55±4.56%)

Table 11. Comparison of Iron Profiles Between Present Study with Bainton-Finch Study

In our study, 27 of 44 thalassaemic cases (61.36%) had serum iron more than 200µg/dL. Erlandson et al in 1964.[22] observed a similar finding, 66.66%. While other studies like that of Cartwright and co-worker and Smith et al observed higher rates of 80% and 82%. This is because of repeated transfusions and also due to increased absorption of iron from intestines.[22]

Swaroop Mitra et al in 1983[19] showed haemoglobin level between 4 and 6, which closely correlates with the present study. The mean haemoglobin concentration in the present studies was 6.31±2.23. Beveridge B.R. et al in 1965[23] studied 371 English patients and got the mean Hb was 7.6. Only eight patients had Hb less than 4 and five patients had Hb more than 11. In the present study, 14 patients had Hb less than 4 and none had Hb more than 11.

In the thalassaemic group, the present study observed that MCV values ranged from 39.01 to 62.61 with a mean of 50.81±11.8. Deborah Rund et al in 1992[24] showed that MCV be 59-80 and J. David Bessman et al in 1983[25] study showed MCV range of 61.2-79.6.

Mean RDW of IDA in this study was 18.27±2.83, which correlated with J. David Bessman et al in 1983[25] the observation of which showed that all patients with IDA had high RDW (>14%).

was in the range of 56.3-87.3. The authors found that in almost all cases of β mutations, MCV was <67, whereas all, but a few β^+ heterozygotes had MCV >67. This implied that MCV value of heterozygotes for β-thalassaemia correlates with the severity of mutations. J. David Bessman et al in 1983[25] found mean MCV in their study to be 70.4±9.2.95.91% of cases have MCV <67.[26]

MCV in fL	Deborah Rund et al in 1992	J. David Bessman et al in 1983	Present Study
	56.3-87.3	61.2-70.6	39.01-62.61

Table 12. Comparative Study With Reference to MCV

The mean MCV in IDA in our present study was 68.72±11.16, this observation is in accordance with that of Bainton Finch in 1964[21] study, which observed MCV range to

	Hb (g/dL)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	RDW (%)
Shafi Mohammad Khosa et al (2015)[13]	7.5±1.2	3.3±0.8	68.9±9.1	20.2±1.9	26.1±2.6	20.4±4.2
Ebrahim Miri-Moghaddam et al (2014)[27]	10.8±1.3	4.8±0.7	72.3±5.8	22.5±3	31±2.2	16.2±2.7
Kook in Park and Kir Young Kim (1987)[28]	9.4±1.7	4.56±0.64	64.2±7.6			
Present study	6.31±2.23	3.51±1.09	68.72±11.16	18.42±4.34	26.95±7.55	18.27±2.83

Table 13. Comparison of RBC Indices among IDA Cases Between Present Study and Other Studies

As evident from the above table, the observations of haematological and iron profile study of IDA in the present study is in the comparable range of three other studies.

In a study done by J. David Bessman et al in 1983[25] to assess the importance of quantitative anisocytosis as a discriminating factor between IDA and thalassaemia minor, 22 of 25 patients with β-thalassaemia had MCV <70f1, RDW

<14%. In IDA, 53 patients with MCV <70 fL, RDW was always >14%. In the present study, 40 out of 44 patients were β-thalassaemic, had MCV <70 fL and RDW is <14, whereas 100 of 166 IDA patients had MCV <70 fL and RDW is >14% in 150 patients of IDA. So, the present study concludes that IDA and βTM are two clinically close differentials and laboratory values like MCV and RDW when evaluated combinedly helps in distinguishing the two with reasonable accuracy. The p value as calculated by Student's T test was significant for both MCV (p <0.001) and for RDW (p<0.001).

Two reasons were suggested in the Bessman study in 1983[25] for the increased anisocytosis as measured by RDW in cases of IDA as compared to βTM. First, iron deficiency results in abnormal erythropoiesis leading to hypochromia, microcytosis, increased variations in size and shape leading to poikilocytosis and anisocytosis. Second, without iron replacement, iron deficiency is progressive rather than stable. But, in thalassaemia minor, the RBC abnormality is not progressive. Therefore, they are exempt from this anisocytosis.

The observations of different studies is compared with the present study as depicted below-

Parameters	Shafi Mohammad Khosa et al (2015)[13]		Ebrahim Miri-Moghaddam et al (2014)[27]		Present study	
	IDA	βTM	IDA	βTM	IDA	βTM
Hb (g/dL)	7.5±1.2	11.5±1.2	10.8±1.3	12.8±1.2	6.31±2.23	8.02±0.96
RBC (10^{12}/L)	3.3±0.8	5.8±0.5	4.8±0.7	7.1±0.5	3.51±1.09	4.52±1.25
MCV (fL)	68.9±9.1	71.4±5.5	72.3±5.8	61±3.1	68.72±11.15	64.63±10.84
MCH (pg)	20.2±1.9	20.2±2.2	22.5±3	18.1±1	18.42±4.34	17.41±4.37
MCHC (g/dL)	26.1±2.6	27.9±2.4	31±2.2	29.7±1.4	26.95±7.55	25.95±6.14
RDW (%)	20.4±4.2	16.0±1.7	16.2±2.7	16.2±1.9	18.31±2.8	13.94±1.55

Table 14. Comparison Between IDA and Thalassaemia in Our Study with Respect to Other Studies

Most of the studies agrees to the finding of decreased red cell parameters with increasing grade of thalassaemias was in our case. The findings of different authors are compared with the present study in a tabular format below for reference.

Parameters	Monica Dogaru et al (2007)[29]		Shanthi G et al (2013)[30]		Present Study	
	β-thal. major	β-thal. minor	β-thal. major	β-thal. minor	β-thal. major	β-thal. minor
Hb (g/dL)	8.6	11.25	4.93	11.26	5.1	6.8
RBC (10^{12}/L)	3.6	4.28	2.6	5.40	2.71	4.52
MCV (fL)	65.8	70.6	65.47	68.52	48.61	64.63
MCH (pg)	23.5	26.24	19.32	20.87	15.61	17.41
MCHC (g/L)	35.7	37.16	29.59	30.62	23.45	25.95

Table 15. Comparative Study of RBC Indices Between Thalassaemia Major and Minor

β-thal major - β-thalassaemia major, β-thal minor - β-thalassaemia minor

CONCLUSION

Anaemia is a commonly encountered clinical problem in southern Odisha especially in the paediatric population. Of the morphological types of anaemia, microcytic hypochromic type is the commonest especially in the developing countries like India. IDA was found to be the most common cause of microcytic hypochromic anaemia followed by thalassaemia as observed by most of the authors.

So, the present study concludes that-

1. CBC is a very cost effective, but powerful diagnostic tool, which can be used for mass screening purpose in cases of anaemia.
2. Though serum iron profile study is diagnostic of IDA, anisocytosis as measured by RDW is the earliest morphological change in IDA.
3. The MCV value is comparatively less decreased in β-thalassaemia minor than IDA.
4. So, evaluation of RDW along with MCV is very useful in differentiating the early IDA from β-thalassaemia minor.
5. Haemoglobin electrophoresis is an easy to perform and cost-effective method for diagnosing various haemoglobinopathies with a reasonable accuracy, but the accuracy is low compared to HPLC.

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