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Clinico-hematological Pattern of Thalassemias and Hemoglobinopathies in Children Presenting with Microcytic Anemia: An Outdoor-based Study at Burdwan, West Bengal

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ABSTRACT

Objectives: Thalassemia and hemoglobinopathies are major causes of microcytic anemia in pediatric age group. The present study was done to calculate the proportion of children suffering from thalassemias and hemoglobinopathies amongst the patients of microcytic anemia and assessment of the clinical and hematological parameters of selected patients.

Materials and Methods: Children of the age group 0-12 years attending the pediatrics outpatient department of Burdwan Medical College who showed microcytic anemia (i.e. MCV less than 80 fl) were included in the study. Among them, those showing features of thalassemia or hemoglobinopathy on HPLC underwent detailed clinical examination and history taking.

Results: 64 (22%) children out of the total 292 patients had thalassemia or hemoglobinopathies. Rest 228 had microcytic anemia due to other causes. Hemoglobin disorders found, in decreasing order of occurrence, were E Beta-thalassemia (45%), Beta thalassemia major (26%), Beta thalassemia trait (17%) and E trait (8%). E homozygous and Beta thalassemia-HPFH had one case each. 42 children had history of blood transfusion. The average age of first transfusion was 14 months for Beta thalassemia major patients and 3 years for E Beta thalassemia patients. The age of first transfusion is highly variable in case of E Beta thalassemia. Hepatomegaly, splenomegaly and skeletal changes were almost exclusively found in transfused children. 56.3 % children suffering from hemoglobin disorders were malnourished; most of them having beta thalassemia major and E beta thalassemia. Beta thalassemia major and E beta thalassemia patients had more severe anemia and anisocytosis than the other hemoglobin disorders which were milder in nature.

Conclusion: Hemoglobin disorders happen to be the cause of ailment in a considerable proportion of children suffering from microcytic anemia. So hemoglobin analysis, preferably by HPLC is very much helpful for early diagnosis and treatment..

Key Words: Children, Hemoglobinopathies, Microcytic anemia, Thalassemia, West bengal

INTRODUCTION

It has been estimated that approximately 7% of the world population are carriers of thalassemia and hemoglobinopathies and that 3, 00,000 –4, 00,000 babies with severe forms of these diseases are born each year [1]. With a population of 1000 million at the millennium year 2000 and approximately 27 million born with pathological hemoglobinopathies each year, India is among the countries worst hit by thalassemia and hemoglobinopathies [2, 3]. The frequency of carriers of hemoglobinopathies varies from 3 to 17% in different population groups of India [4]. The cumulative gene frequency of the three most predominant abnormal hemoglobins, i.e. sickle cell, hemoglobin D and hemoglobin E has been estimated

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to be 5.35% in India [5].

The abnormal hemoglobins so far detected in India include Hb D, E, H, J, K, L, M, Q, S, Lepore, Norfolk, Koya Dora, Chandigarh and the hereditary persistence of HbF.

The distribution of different thalassemias and hemoglobinopathies show remarkable variation in different parts of the country and in different ethnic and tribal population [6].

The most commonly found abnormal hemoglobins in India are sickle cell hemoglobin (S), hemoglobin-E and hemoglobin-D.

In the very few studies done in West Bengal, mainly in adult population- based study or prevalence based study during pre-marital screening, β-thalassemia was found to be most prevalent Hb disorder with β-thalassemia carriers in the range 3.5% to 10% [7, 8]. HbE comes second with carrier about 4.5% [7].

Most thalassemias and hemoglobinopathies produce anemia with smaller i.e. microcytic RBCs (red blood cells) and thus are grouped together with other causes of microcytic anemia, notably iron deficiency anemia, anemia of chronic disorders and sideroblastic anemia. Microcytic hypochromic anemia is fairly common and significant microcytosis is detected in nearly 3% of all patients who require admission to the hospital [9].

While similar in many respects, the management of these different conditions is also very different. There are very few studies in pediatric population regarding microcytic hypochromic anemia and there are not much studies in Indian population.

This study was undertaken to quantify the proportion of patients coming to the pediatric outpatient department suffering from one or the other form of thalassemia or hemoglobinopathies and to assess their clinico- hematological status.

MATERIAL AND METHODS

This study was conducted at the Outpatient department, Burdwan Medical College & Hospital and at the Department of Pathology, Burdwan Medical College for a period of one year (February, 2010 to January, 2011). Convenience sampling was done amongst the patients suffering from anemia in the age group 0-12 years from a rural area around Burdwan. The patients who showed microcytic RBCs i.e. Mean red cell volume (MCV) less than 80 fl on analysis by automated cell counter were included in the study.

First of all, history regarding age, sex, religion, address, family history of any illness was taken. They were inquired for onset of symptoms, weakness or lethargy, poor feeding, jaundice, recurrent fever, cough and cold, diarrhea, painful crises and ulcers. History of blood transfusion and splenectomy also were taken. Height and weight of the patients were measured and plotted in the IAP growth chart for assessment of status of growth [10]. Clinical examination was done regarding; pallor, jaundice, facies, skin and systemic examination with special emphasis to liver and spleen enlargement.

3 ml of EDTA blood and 2 ml of unanticoagulated blood was collected from each patient. From the EDTA blood Hemoglobin level was estimated by cyanmethemoglobin method. Total count of RBC, Red cell indices i.e. Hematocrit (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), Total count of WBC and platelet count was measured using SYSMEX KX21 automated blood cell counter. A Leishman stained peripheral blood smear was prepared for differential leukocyte count and corroboration of findings of automated counter. Reticulocyte count was done after supravital staining using new methylene blue stain.

High performance liquid chromatography (HPLC) for Hemoglobin variants was done using BIORAD variant hemoglobin testing system. Manufacturer guidelines were followed for interpretation of HPLC. As per ICMR guidelines, the cut-off for HbA2 value for detection of heterozygous β-thalassemia was kept at 4.0%

RESULTS

Of the total 292 patients having microcytic anemia included in the study, 64 (22%) had thalassemia or hemoglobinopathies. Rest 228 had microcytic anemia due to other causes. In the thalassemia or hemoglobinopathy group, 35 (54.7%) were male and 29 (45.3%) were female.

Table 1: Age-sex distribution of patients

Age	Number of cases	Male	Female
< 3 months	0	-	-
3-6 month	0	-	-
6 month- 1 yr.	0	-	-
1-4 yr.	28	14	14
4 yr.- 12 yr	36	21	15

All patients included in the study were of the age 1 year or above with median of 4 yrs 8 months; the youngest 13 months old and the oldest 11 years old. (Table 1)

Children even with a single transfusion were included in the "Transfused" group and others were termed as "Untransfused". From table 3 it is seen that all the Beta thalassemia major patients and most of the E Beta thalassemia patients required blood transfusion. All the other patients did not require blood transfusion.

Table 2: Relative proportions of different thalassemia and hemoglobinopathies

Beta thal major	Beta thal trait	E Beta	E trait	E homozygous	Beta thal-HPFH
17	11	29	5	1	1

Table 3: Blood transfusion status

	Transfused	Untransfused	Total
Beta thal major	17 (100%)	0	17
Beta thal trait	0	11 (100%)	11
E Beta thal	25 (86.2%)	4 (13.8%)	29
E trait	0	5 (100%)	5
E homozygous	0	1 (100%)	1
Beta thal HPFH	0	1 (100%)	1
Total	42 (65.6%)	22 (34.4%)	64

Table 4: Initiation and interval of blood transfusion

	Blood transfusion since (age in months)		Interval of blood transfusion (months)	
	Mean	Median (Range)	Mean	Median (Range)
Overall	28.1	18.5 (3–96)	1.8	1 (0.5–6)
Beta thal major	13.6	14 (6–24)	1.4	1 (1–2)
E Beta thal	37.9	30 (3–96)	2.1	1 (0.5–6)

Table 5: Clinical findings

			TRANSFUSION STATUS		NATURE OF THALASSEMIA AND HEMOGLOBINOPATHIES					
		Overall	Transfused	Untransfused	Beta thal major	Beta thal trait	E beta	E trait	E homozygous	Beta thal HPFH
PALLOR	PRESENT	56	42	14	17	8	29	1	0	1
	ABSENT	8	0	8	0	3	0	4	1	0
	%	87.5	100.0	63.6	100.0	72.7	100.0	20.0	0.0	100.0
	TOTAL	64	42	22	17	11	29	5	1	1
JAUNDICE	PRESENT	10	10	0	4	0	6	0	0	0
	ABSENT	54	32	22	13	11	5	1	1	1
	%	15.6	23.8	0.0	23.5	0.0	20.7	0.0	0.0	0.0
	TOTAL	64	42	22	17	11	29	5	1	1
HEPATOMEGALY	PRESENT	33	33	0	14	0	19	0	0	0
	ABSENT	31	9	22	3	0	10	5	1	1
	%	51.6	78.6	0.0	82.4	0.0	65.5	0.0	0.0	0.0

	TOTAL	64	42	22	17	11	29	5	1	1
SPLENOMEGALY	PALPABLE	33	32	1	10	1	22	0	0	0
	NOT PALPABLE	19	8	21	6	10	6	5	1	1
	SPLENECTOMIZED	2	2	0	1	0	1	0	0	0
	%	51.6	76.2	4.5	58.8	9.1	75.9	0.0	0.0	0.0
	TOTAL	64	42	22	17	11	29	5	1	1
SKELETAL CHANGE	PRESENT	17	17	0	6	0	11	0	0	0
	ABSENT	47	25	22	11	11	18	5	1	1
	%	26.6	40.5	0.0	35.3	0.0	37.9	0.0	0.0	0.0
	TOTAL	64	42	22	17	11	29	5	1	1
NUTRIONAL STATUS	MALNOURISHED	36			13	2	20	0	0	1
	%	56.3			76.5	18.2	69.0	0.0	0.0	100.0
	TOTAL	64			17	11	29	5	1	1

- Clinically detectable pallor was present in all of the children having blood transfusion and majority of the children not having transfusion.
- Most of the children with Beta thalassemia trait had pallor but most of the E trait children showed no pallor.
- Only mild icterus was noticeable if at all present and all icteric children were on blood transfusion.
- Clinically detectable enlargement of liver and spleen was mostly a monopoly of the children on blood transfusion; only one child without transfusion (diagnosed Beta thalassemia trait) showed mild splenomegaly.
- 82% Beta thalassemia major patients and 66% E Beta thalassemia patients had hepatomegaly.
- 59% Beta thalassemia major patients and 76% E Beta thalassemia patients had splenomegaly.
- Splenectomy was done in one patient each with Beta thalassemia major and E Beta thalassemia.
- Skeletal changes characteristic of hemoglobin disorders i.e. frontal bossing, undue prominence of malar eminence and dental malocclusion were observed in about one in three children on blood transfusion; no children without transfusion showed these changes.
- 35% Beta thalassemia major patients and 38% of E Beta thalassemia major patients showed prominent skeletal changes.

Nutritional status of thalassemic patients

Nutritional status was assessed according to the Harvard standard, expressed as percentiles with respect to height (or length as applicable) and weight. A children having body weight less than 80% of the 50th percentile was termed "malnourished".

- Most of the Beta thalassemia major and E Beta thalassemia patients were malnourished.
- Only 18.2% of Beta thalassemia trait patients were malnourished.
- E trait and E homozygous patients did not have malnutrition.
- The Beta thal-HPFH patient was malnourished.

Evaluation of laboratory parameters

For the purpose of brevity some parameters will have to be mentioned in abbreviated form as follows:

- Hb – Hemoglobin concentration in g/dl
- TCRBC – Total count of RBC in $1x10^6/\mu l$
- PCV – Packed cell volume in percentage.
- MCV – Mean red cell volume in femtolitre (fl) i.e. $1x\ 10^{-15}$litre
- MCH –Mean corpuscular hemoglobin in pictogram (pg) i.e. 1x10-^{12}litre
- MCHC – Mean corpuscular hemoglobin concentration in g/dl
- RDW (CV) – Red cell distribution width (coefficient of variation) in percentage
- TLC – Total leukocyte count/ µl
- NRBC –Number of nucleated red cells expressed in percentage of total nucleated cells in peripheral blood smear
- Retic (corrected) – Reticulocyte count expressed in percentage of all red cells in peripheral blood corrected for degree of anemia.
- HbF – Fetal hemoglobin expressed in percentage of total hemoglobin
- HbA – Normal adult hemoglobin expressed in percentage of total hemoglobin
- HbA_2 – Hemoglobin A_2 expressed in percentage of total hemoglobin
- HbE – Hemoglobin E expressed in percentage of total hemoglobin
- Overall the patients were grossly anemic with reduced TCRBC, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and very high RDW (CV).

Table 6: Hematological parameters (overall)

	Hb	TC RBC	PCV	MCV	MCH	MCHC	RDW(CV)
Mean	6.87	3.45	22.80	67.39	20.46	29.61	26.60
Std. Dev.	2.28	1.25	7.54	6.71	3.30	2.95	8.60

Table 7: Hematological findings of Thalassemia and hemoglobinopathies patients

Disease Groups		Hematological Parameters									
		Hb	TC RBC	PCV	MCV	MCH	MCHC	RDW(CV)	TLC	NRBC	Retic (corrected)
Beta thalassemia major	Mean	5.41	2.43	17.15	70.15	22.4	29.71	29.66	7665	18.23	1.57
	Std. Dev.	1.78	0.83	6.17	6.13	3.22	3.42	8.79	2063.1	9.81	0.18
Beta thalassemia trait	Mean	9.17	4.91	30.66	63.39	19.04	29.79	17.8	8009	0	1.2
	Std. Dev.	1.61	1	5.12	6.21	2.68	1.74	2.89	1591.5	0	0.33
E Beta thalassemia	Mean	6	3.2	20.77	66.75	19.32	28.77	30.99	8555	15.4	1.61
	Std. Dev.	1.25	0.82	4.46	6.75	2.87	2.72	5.3	2815.6	10.8	0.23
E trait	Mean	10.7	4.93	34.68	70.5	22.14	31.54	14.28	9160	0	1.08
	Std. Dev.	0.49	0.21	1.58	4.71	1.88	1.2	0.6	1597.8		0.43
E homozygous		10.6	5.42	32.4	59.8	19.6	32.7	16.4	10400	0	0.7
Beta thal –HPFH double heterozygous		8.4	2.94	22.2	75.2	28.4	37.8	15.7	11200	2	0.5

- Beta thalassemia major and E Beta thalassemia patients showed severe degree of microcytic anemia with marked anisocytosis. Numerous nucleated red cells were seen.
- Beta thalassemia trait subjects had mild to moderate anemia with severe microcytosis but mild anisocytosis.
- E trait subjects had mild anemia with unremarkable associated features.
- E homozygous patient had mild anemia with slightly raised RDW (CV).
- Beta thal –HPFH double heterozygous patient showed moderate anemia with other parameters consistent with the degree of anemia. RDW (CV) was slightly higher than normal. A few nucleated red cells were seen. Corrected reticulocyte count was within normal range.
- Beta thalassemia trait, E trait and E homozygous patients did not have nucleated red cells in peripheral smear.
- Interestingly, corrected reticulocyte count was within normal range in all the patients.

Hemoglobin analysis by HPLC

Table 8: HPLC characteristics of thalassemia and hemoglobinopathies

		HbF	HbA_2	HbA_0
Beta thalassemia major	Mean (SD)	44.38(29.26)	3.84 (1.29)	47.28 (24.47)
Beta thalassemia trait	Mean (SD)	1.38 (1.37)	4.86 (0.42)	83.78 (2.8)
		HbF	HbA_2+HbE	HbA_0
E Beta thalassemia	Mean (SD)	22.12(13.32)	42.22(16.58)	28.28 (19.76)
E trait	Mean (SD)	0.62 (0.39)	24.14 (3.64)	64.58 (3.29)
E homozygous (single patient)		3.3	77.7	4.8
		HbF	HbA_2	HbA_0
Beta thalassemia-HPFH double heterozygous (single patient)		97.5	1.3	2.3

All the thalassemic and hemoglobinopathic children showed usual features on HPLC for hemoglobin variants. Notably all Beta thalassemia trait subjects had HbF in the normal range and HbA_2 well above the cut-off of 4%.

There was extreme elevation of HbF with HbA2 lying in the normal range in the Beta thalassemia – HPFH double heterozygous patient. He was diagnosed on clinical grounds with the aid of parental screening which showed one parent having beta thalassemia trait and the other with HPFH heterozygous.

Table 9: HPLC characteristics of microcytic anemia not due to hemoglobin disorders

Type of microcytic anemia					
Non-hemoglobinopathic		Mean	Std. Dev.	Min.	Max.
	HbF	0.742	0.672	0	3.8
	HbA_2	2.50	0.274	1.8	3.2
	HbA	87,46	1.578	78.7	91.1

All of these children had HPLC showing normal hemoglobin analysis with Fetal Hemoglobin and HbA_2 in the normal range. HbA_2 values were in the range 1.8% to 3.2%.

Table 10: Comparison of the thalassemic group (64) and non-thalassemic group (228)

	Thalassemic (64)		Non-thalassemic (228)		Result of two-tailed independent t-test	
	Mean	Std. Dev.	Mean	Std. Dev.	t-value (290 df)	P value
Age (months)	59.4	36.5	46.4	32.1	2.761	0.006
Hb	6.87	2.28	9.30	1.75	-9.180	<0.001
TC RBC	3.45	1.25	4.45	0.71	-8.215	<0.001
PCV	22.80	7.54	31.73	5.02	-11.150	<0.001
MCV	67.39	6.71	71.60	5.80	-4.949	<0.001
MCH	20.46	3.30	21.01	2.75	-1.351	0.178
MCHC	29.61	2.95	29.23	2.09	1.174	0.241
RDW(CV)	26.60	8.60	18.31	3.90	11.071	<0.001
TLC	8342.3	2362.7	9918.4	3705.6	-3.222	0.001

- The children with hemoglobin disorders showed significantly more severe degree of anemia and higher degree of anisocytosis than the non-thalassemic children.
- The non-thalassemic children show higher leukocyte counts than the thalassemic children. Both the mean values are within normal range.

DISCUSSION

The present study included total 292 children of the age group 0-12 years. Hemoglobin disorders constituted 22% of the cases. In earlier studies by Derkjen van Zeben et al [11] among patients of all age groups and by Martín Núñez G et al [12] among school children, the proportion of microcytic anemia was about 10%. In their studies they did not include the patients on regular blood transfusion or on iron supplementation. The difference in inclusion criteria may be the cause of increased proportion of hemoglobin disorders in the present study. Manna AK et al [7] in 2009 and Basu et al [13] in 2002 observed incidence of thalassemia and hemoglobinopathies in to be 25.15% and 20.42% respectively during clinic based screening in and around Kolkata, West Bengal, India for these disorders in all age groups. However they did not mention the exact proportion among patients with microcytic anemia.

In this study E Beta-thalassemia (45%) was found to be the most frequent among the hemoglobin disorders (Table 2) followed by Beta thalassemia major (26%), Beta thalassemia trait (17%) and E trait (8%) whereas Basu et al [13], in their study in Kolkata and adjoining suburbs in 2002, observed Beta thalassemia trait (68%) as the most commonly occurring hemoglobin disorder followed in order by HbE heterozygous (25%), E Beta-thalassemia (18%) and Beta thalassemia major (6%). Manna AK et al [7] reported Beta-thalassemia minor (44%) as the single-most abnormal population around Kolkata followed by E Beta-thalassemia (22%), HbE-heterozygous (18%), Beta-thalassemia major (12%).

The present study show fewer number of Beta thalassemia trait patients than in the previous studies probably because it did not include the adult patients coming for voluntary premarital screening which comprises of a major portion of people opting for hemoglobin analysis. In both past studies E-Beta thalassemia emerged as a greater threat than other symptomatic hemoglobin disorders which is in strong agreement with the present study.

When analyzing the age of first blood transfusion and interval between successive transfusions (Table 4), it was found that Beta thalassemia major patients were transfused from a mean age of 6-24 months (mean 13.6 months) at an interval of 1-2 months (mean 1.4 months) and the transfusion dependent E Beta thalassemia patients were transfused from age of 3months to 8 years (mean 37.9 months) at an interval of 15 days to 6 months (mean 2.1 months). In an ethnically composite population of transfusion-dependent Beta thalassemia patients diagnosed in the United Kingdom, the mean age at presentation was reported to be 6 months; in a study from Greece, the age was 13.1 months, ranging from 2 months to 3years [14, 15]. The present study is consistent with these reports as far as age of first transfusion is concerned. Our study also reflects the high degree of clinical variability

of E Beta thalassemia ranging from severe transfusion dependent anemia to milder forms and thus conforms to earlier studies by Fucharoen, S et al [16].

A brief assessment of growth status of the children with hemoglobin disorders with respect to height and weight was done in the present study (Table 5) which showed that malnutrition was present in 76.5% of Beta thalassemia major patients and 69.0% E Beta thalassemia patients. On the contrary, only 18.2% of Beta thalassemia trait patients were malnourished while E trait and E homozygous patients were well-nourished. From this observation it follows that patients with severe transfusion dependent anemia were more often malnourished than the non-transfusion dependent children. The more elaborate evaluation using growth charts also reflects that height and weight of E Beta thalassemia patients were dispersed more heterogeneously than the Beta thalassemia major patients further confirming the great clinical variability of E Beta thalassemia. The retardation of growth occurs as a consequence of anemia responsible for tissue hypoxia. Tissue hypoxia stimulates secretion of Erythropoietin which in turn causes expansion of the dyserythropoietic marrow. This causes diversion of calories required for normal development to the ineffective red cell precursors. So severely affected patients show poor development and wasting [17].

On comparing the two groups of children having microcytic anemia i.e. the children having hemoglobin disorders and children without it, it has been observed that the thalassemic children had more severe degree of anemia. The presence of patients with severe transfusion-dependent anemia in the first group explains this difference with the latter. The degree of anisocytosis was also higher in the thalassemic children. As mentioned earlier, the transfusion dependent disease conditions namely the Beta thalassemia major and E Beta thalassemia showed very high degree of anisocytosis reflected by the high values of RDW (CV). No literature expressing definitive opinions regarding degree of anisocytosis in Beta thalassemia major and E Beta thalassemia patients could be found, but it appears that it may be due to the presence of multiple populations of red cells in the transfusion dependent patients that aggravates the dispersion of red cell volume. Detailed study incorporating more number of patients is required to investigate the source of variation of red cell size in these disorders.

The significantly higher value of leukocyte count of the non-thalassemic children is probably incidental or may be due to the patients with microcytic anemia associated with inflammation.

CONCLUSION

A significant part of the children presenting with microcytic anemia in the outpatient department are suffering from thalassemia or hemoglobinopathy. So hemoglobin analysis, preferably by HPLC is very much helpful when clinical features of hemoglobin disorders are not apparent.

E beta thalassemia and beta thalassemia major were found to be the prominent culprits in this study. It is evident from the present study and many previous studies that thalassemia and hemoglobinopathies need greater focus in terms of population based studies, mass education, premarital counseling, early diagnosis and nutritional interventions.

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