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VARIATION IN LEVELS OF HbA2 AND RBC INDICES WITH SPECIAL REFERENCE TO COMMON HEMOGLOBINOPATHIES REPORTED IN TERTIARY HEALTH CENTRE

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ABSTRACT

Variation in levels of HbA2 is constantly associated with various hematological and non-hematological conditions. Though exceptional elevation in levels of HbA2 is one of the most significant parameters in diagnosing beta thalassemia carrier. But, in spite of RBC indices favoring diagnosis of the same, presence of other associated hematological or non-hematological conditions can present with either increase or even decrease in HbA2 levels upon capillary electrophoresis. Similar disparity can also be seen during quantification of HbA2 in other hemoglobinopathies by capillary electrophoresis. The aim of present study is to correlate the levels of HbA2 (as determined by capillary electrophoresis) with RBC indices in commonly encountered hemoglobinopathies, taking non hematological conditions into consideration too. In the study period of 12 months, 109 selected cases are referred for capillary electrophoresis in our Department of Pathology. Complete blood count and peripheral blood smear study and capillary electrophoresis is done in selected cases. Values of RBC indices with HbA2 levels are tabulated for comparison. β-thalassemia minor and α- thalassemia trait are most common hemoglobinopathies with 25 cases (22.9%) of each followed by β-thalassemia major with 16 cases (14.7%). β-thalassemia minor being asymptomatic is seen most commonly in 3rd decade (14 cases) of life. Male to female ratio is found to be 2:1. Due to associated haematological and non haematological conditions HbA2 levels vary deviating from expected ranges. Thus, only HbA2 levels cannot be relied upon as such for diagnosis of hemoglobinopathies.

Keywords: β thalassemia, HbA2, Capillary electrophoresis, RBC indices.

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INTRODUCTION

HbA2 is a minor component of RBC, comprising of two alpha and two delta chains constituting less than 3% of total hemoglobin [1]. For diagnosing beta thalassemia trait determination of HBA2 level plays an important role [2, 3]. Majority of thalassemia trait patients do not require special therapy or other treatment modalities; still genetic counselling of carrier states can prevent impending major disease in children [4].

Among RBC indices presence of microcytosis, hypochromia and normal or increased RBC count along with increased level of HbA2 should warrant laboratory screening for thalassemia trait. But few cases of trait can have normal HbA2 level as some are not associated with elevated levels of HbA2 or can have associated iron deficiency or alpha thalassemia [1, 3].

Quantitative analysis of HbA2 can be quite cumbersome as its value is very less in normal conditions and increases only slightly in diseases. Procedures with

high accuracy or precision are thus preferred as some other types of hemoglobins can also interfere in its estimation. For measurement of HbA2 cation-exchange high-performance liquid chromatography (HPLC), microcolumn chromatography, and cellulose acetate electrophoresis with elution were employed [5].But, with more precise estimation of its level by capillary zone electrophoresis (CZE), this method of automated electrophoresis is emerging as a powerful technique. The separation of protein is achieved in a liquid buffering system running through parallel, narrow-bored capillaries under high-voltage. Hemoglobins are measured at a certain wavelength. This method has the advantages of high efficiency, high accuracy, and full automation. Also several other types of hemoglobins can be segregated quantitively.

The aim of the study is to emphasise on variation in levels of HbA2 levels from normal expected ranges due to associated haematological and non haematological conditions.

MATERIALS AND METHODS

This study is conducted in Department of Pathology, Silchar medical college and hospital from March,2016 to February, 2017.Cases are taken after evaluation of reports of CBC (Complete blood count) and peripheral blood smear. 109 selected cases are referred for capillary electrophoresis. Reports of electrophoresis are interpreted according to the described standard range.

In this study, electrophoresis is done by Sebia CAPILLARYS 2 FLEX-PIERCING System. In this system, CAPILLARYS HEMOGLOBIN(E) kit demonstrates the separation of the normal hemoglobin (A, A2 and F) in human blood samples, and the detection of the major hemoglobin variants (S, C, E and D), by capillary electrophoresis in alkaline buffer (pH 9.4). The CAPILLARYS 2 FLEX-PIERCING System with the CAPILLARYS HEMOGLOBIN(E) kit is designed for laboratory use.

The CAPILLARYS 2 FLEX-PIERCING is an automated analyzer which performs a completed hemoglobin profile for the quantitative analysis of the normal hemoglobin fractions A, A2 and F and for the detection of major hemoglobin variants S, C, E and D. The assay is performed on the hemolysate of whole blood samples collected in tubes containing EDTA as anticoagulant. The measurement of the percent of HbA2 and HbF is effective in the diagnosis of thalassemias (i.e. hereditary hemolytic anemias characterized by decreased synthesis of one or more types of normal hemoglobin polypeptide chains) and this technique allows the detection of the major hemoglobin variants that are caused by different genotype combinations depending upon the geographical area.

To rule out various hemoglobinopathies, value of HbA2 >3.4% is considered as increased level while <2.2 % as decreased one with normal range of HbA2 as 2.2% to 3.2%.

RESULTS

In total, 109 patients Hb electrophoresis and corresponding RBC indices are correlated in this study period. β-thalassemia minor and α- thalassemia trait outnumbered all other hemoglobinopathies with a total of 25 cases (22.9%) of each followed by β-thalassemia major with 16 cases (14.7%). β-thalassemia minor being asymptomatic in majority is seen most commonly in 3rd decade (14 cases) of life. α-thalassemia trait and beta thalassemia major are seen most commonly in 1st decade with a total of 11 cases and 12 cases, respectively. Cases of mixed hemoglobinopathies of HbE and β- thalassemia counts for total of 3 cases only (Table 1).

Male to female ratio is found to be 2:1. For almost all hemoglobinopathies there is male preponderance but for beta thalassemia major, females(9cases) are more affected than males (7cases).(Table 2).

When RBC indices are compared along with HbA2 levels, highest level of HbA2 is seen in sickle cell anemia (3.95%) with minimum RBC count ($2.94x10^6/\mu L$), Hct (22.9%) and Hb (6.6g/dL). This was followed by combined hemoglobinopathy of sickle cell and β-thalassemia with HbA2 level of 3.9 %. All other RBC indices are more than in SCT or SCA except MCH(21.4pg), MCHC (29.7g/dL) and RDW (15.3%). (Table 3)

For β-thalassemia minor HbA2 is slightly less (3.18%) than that for β-thalassemia major (3.26%). Though RBC count and Hb is more in minor disease than major ($4.80 \times 10^6/\mu L$vs $3.29x10^6/\mu L$) and (9.9 g/dLvs 8.0g/dL) respectively, all other RBC indices are increased in major (MCV- 65fL vs 70fL; MCH – 20.8 pgvs 23.8 pg ; MCHC- 31.5g/dLvs 33.8g/dL ; RDW-16.2% vs 16.5 %). (Table 3)

With α-thalassemia, low levels of HbA2 (2.20%) are seen but all other RBC indices are more towards normal (MCV – 74.7fL; MCH- 24 pg ; MCHC – 33.1g/dL ; RDW-15.5 %) . RBC indices for HbE trait (MCV-83.4fL; MCH-26.7pg; MCHC – 32g/dL; RDW- 15.4%), HbE disease (MCV – 71.4fL; MCH- 21.0pg; MCHC- 29.4 g/dL; RDW- 16.3%) and HbE /β-thalassemia (MCV-64.0fL; MCH- 20.0 pg; MCHC- 30.6 g/dL; RDW-25 %)are not severely altered but HbA2 for combined HbE /β-thalassemia is more (3.20%) than that for HbE trait (1.21%) and HbE disease (1.95%) (Table 3).

Table 1. Age distribution of cases

	0 – 10	11 – 20	21 - 30	31 – 40	>40	Total
β-thalassemia minor	03	05	14	03	-	25
β-thalassemia major	12	03	01	-	-	16
α-thalassemia trait	11	10	03	01	-	25
HbE trait	-	01	02	04	03	10
HbE disease	-	03	04	05	02	14
HbE/ β-thalassemia	02	01	-	-	-	03
Sickle cell trait	-	02	01	02	-	05
Sickle cell anemia	02	02	01	-	-	05
Sickle/ β-thalassemia	02	-	02	01	01	06
	32	27	28	16	06	109

Table 2. Sex distribution of cases

	Male	Female	Total	Percentage (%)
β-thalassemia minor	15	10	25	22.9
β-thalassemia major	07	09	16	14.7
α-thalassemia trait	19	06	25	22.9
HbE trait	08	02	10	09.2
HbE disease	10	04	14	12.8
HbE/ β-thalassemia	02	01	03	02.8
Sickle cell trait	03	02	05	04.6
Sickle cell anemia	04	01	05	04.6
Sickle/ β-thalassemia	04	02	06	05.5
	72	37	109	100

Table 3. Hematological features of different hemoglobinopathies

	RBC ($x10^6$/L)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	HbA2 (%)
β-thalassemia minor	4.80	09.9	31.8	65.0	20.8	31.5	16.2	3.18
β-thalassemia major	3.29	08.0	23.0	70.0	23.8	33.8	16.5	3.26
α-thalassemia trait	4.20	10.4	31.4	74.7	24.0	33.1	15.5	2.20
HbE trait	4.60	12.3	38.4	83.4	26.7	32.0	15.4	1.21
HbE disease	4.90	10.3	35.0	71.4	21.0	29.4	16.3	1.95
HbE/ β-thalassemia	4.50	08.9	29.0	64.0	20.0	30.6	25.0	3.20
Sickle cell trait	3.60	09.5	31.0	66.0	23.6	29.8	19.0	3.80
Sickle cell anemia	2.94	06.6	22.9	77.9	22.4	28.8	23.1	3.95
Sickle/ β-thalassemia	4.70	10.1	34.0	74.0	21.4	29.7	15.3	3.90

Table 4. Different causes of variation of HbA_2

HbA2	Inherited	Acquired
Increased (> 3.4 %)	Deletional HPFH from Vietnamese/South East Asian	Thyrotoxicosis
	Hereditary high HbA2	HIV infection
	Unstable hemoglobin	Zidovudine therapy
	β-Thalassemia minor	Megaloblastic anemia (some cases)
	Sickle cell trait	
	Sickle cell anemia (particularly coexisting α-thalassemia)	

	HbS/ β^0-thalassemia	
	Congenital dyserythropoietic anemia (some cases)	
	Heterozygosity for other β-chain variants	
Decreased (< 2.2 %)	Deletional HPFH (except Vietnamese/South East Asian type)	Severe iron deficiency
	α-thalassemia: α^+homozygosity, α^0heterozygosity and HbH disease	Anemia of chronic disease
	δβ and $^A\gamma\delta\beta$-thalassemia heterozygosity (some cases)	Sideroblastic anemia
	δ-Thalassemia	Lead poisoning
	Hemoglobin Lepore	Juvenile myelomonocytic leukemia
	Hemoglobin Kenya	Acquired Hb H disease
		Acute myeloid leukemia (some cases, particularly erythroleukemia)
		Aplastic anemia (some cases)
		Hypothyroidism
		Chemotherapy- induced increased Hb F synthesis

Table 5. Altered mean HbA2 level in various hemoglobinopathies with associated condition

Sl. No.	Disease Condition	Total Number of cases	Number of cases With associated condition	Mean HbA_2(%)
1	β-thalassemia minor	25	17	3.18
2	β-thalassemia major	16	08	3.26
3	α-thalassemia trait	25	15	2.20
4	HbE trait	10	04	1.21
5	HbE disease	14	09	1.95
6	HbE/ β-thalassemia	03	01	3.20
7	Sickle cell trait	05	_	3.80
8	Sickle cell anemia	05	01	3.95
9	Sickle/ β-thalassemia	06	_	3.90

Fig 1

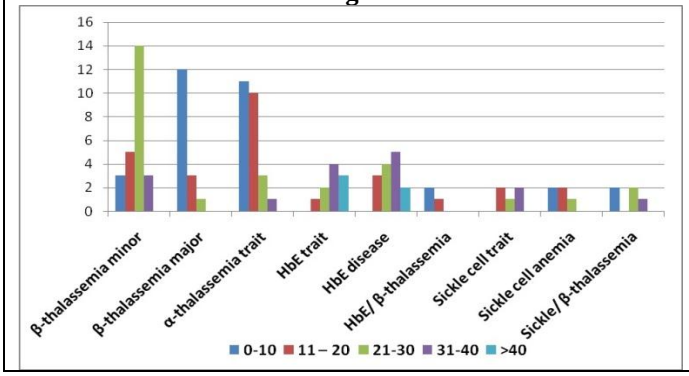


Fig 2

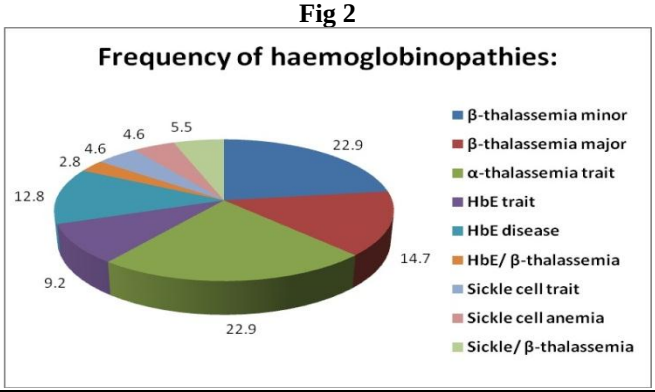
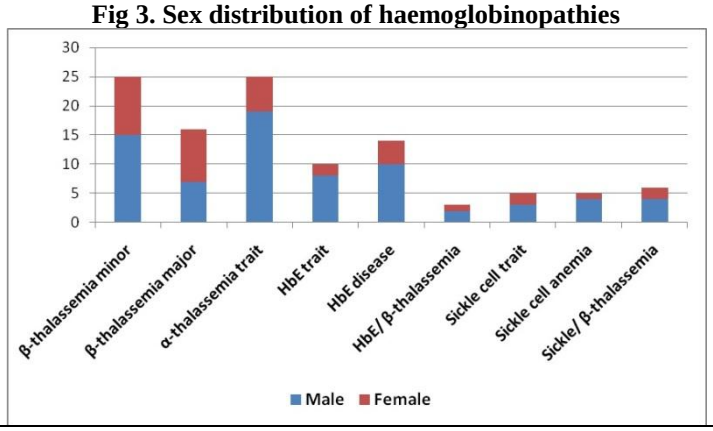


Fig 3. Sex distribution of haemoglobinopathies



DISCUSSION

Characteristically, microcytosis in peripheral blood smear and increased levels of HbA2 levels points towards diagnosis of β- thalassemia trait. But sometimes normal HbA2 levels can also be seen in these cases due to some associated conditions with low HbA2 levels and some other hemoglobinopathies [1, 2, 3-6]

There are many inherited and acquired factors that interfere in HbA2 levels as shown in the Table 4 [3, 4]. Likewise, in this study mean HbA2 levels are altered from the expected values in hemoglobinopathies due to these conditions Table 5.

Hemoglobinopathies frequently encountered in India include β-thalassemia, HbE /β-thalassemia, HbE trait and HbE disease, sickle cell anemia and α- thalassemia. In north east region of India particularly, HbE and sickle cell anemias are frequently encountered. In this study, β-thalassemia minor and α- thalassemia are most common hemoglobinopathies followed by β-thalassemia major, HbE disease, HbE trait, Sickle/ β-thalassemia, Sickle cell trait, Sickle cell anemia and HbE/ β-thalassemia.

In study of Goswami *et al*, Hb E trait was the most common hemoglobinopathies (34.4%) followed by homozygous E (25.3%), β-thalassemia trait (17.8%), E-β-thalassemia (15.1%), β-thalassemia major (1.5%), sickle cell-β-thalassemia (3.4%), sickle cell trait [7].In study done by Patel U et al in population of Gujarat, beta Thalassemia trait was most common hemoglobinopathy, followed by Thalassemia major, sickle cell anemia and sickle cell trait [8].

HbE occurs at an extremely high frequency in many Asian countries. Coin herited HbE beta thalassemia occurs frequently due to variation in frequency of beta thalassemia alleles[8]. But in our study also 2.8% abnormal hemoglobins were HbE beta Thalassemia. Due to non-availability of molecular analysis, diagnosis was made by combination of electrophoresis findings and by screening of parents for thalassemia and HbE. In these patients absent HbA band and increased HbF and HbA2 were present. In one case one, parent was Beta thalassemia trait and other was Hb E trait. These patients had lower mean hemoglobin and red cell indices than HbE homozygous and HbE trait. Since this study concentrated on cases with abnormal electrophoresis findings, low number of HbE cases might be reported. Since these patients are asymptomatic, they may not have presented to hospital or may not have been referred for electrophoresis. The Hb E trait patients were also asymptomatic patients, so their electrophoresis being run as part of family screening of patients having abnormal electrophoresis.

In our study mean hemoglobin levels in HbE beta thalassemia is 8.9g/dL. The heterozygous state for HbE is characterized by minimal morphological abnormalities of the red cells and normal red cell indices; HbE constitutes 25%–30% of the hemoglobin. Homozygotes for HbE have hypochromic microcytic red cells with significant morphological abnormalities including increased numbers of target cells. They are mildly anemic and the overall hematological findings are very similar to those of heterozygous β-thalassemia [6]. Here red cell indices for HbE trait were normal to slightly microcytic hypochromic and Red cell distribution width (RDW) had a Mean of 15.4%. There were 14 cases of HbE homozygous in our study. These patients have mean hemoglobin of 10.3 gm/dl but showed moderate anisocytosis and low MCV and MCH. Mean RDW was16.3%.

Differentiation of HbE disease beta thalassemia from homozygous HbE in samples containing HbA2/E > 75% and HbF< 15% is difficult.In places where the molecular analysis is not available, HbF> 5% in combination with MCV < 55 fL and hemoglobin < 10 g/ could be used for screening of β-thalassemia/HbE disease [9].

Mean MCV for HbE beta thalassemia was 60fL. Although molecular analysis is not used for diagnosis in our study, these patients were symptomatic, had moderate to severe anemia and their one parent had β- thalassemia trait.Fifty-three percent were male and 47% were females in study of Manan [10]. Similarly, Yagnik and Balgir reported 65.5, 56 and 62.1% of male patients, respectively [11, 12]. In our study, also 66% patients were males. As suggested by Manan et al, this might be due to the gender

bias among the parents of these ill children who seek medical care and are ready to spend more for their male children only.

In study of Mehdi SR MCV and MCH were very low in cases of thalassemia presenting microcytic hypochromic picture on peripheral blood smear, however, these values were within the normal limits in sickle cell disorders. The red cell count was increased in cases of thalassemia while it was not much affected in sickle cell disorders. In another study, Mehadi et al also concluded that moderate degree of microcytosis (MCV ≤ 78fl) and hypochromia (MCH ≤ 27pg) was a feature of β thalassemia trait and homozygous α-thalassemia. However, microcytosis was more marked in β thalassemia trait compared to heterozygous α-thalassemia.

In our study, the mean hemoglobin as well as RBC count was lowest for SCA (6.6 gm/dl), followed by beta thalassemia major (8 gm/dl). Sickle cell anemia patients had lower mean hemoglobin level than beta thalassemia traits (6.6 gm/dl vs 9.9 gm/dl). While RBC count was very less in sickle cell anemia (mean 2.94 million/cumm), it was more towards normal in case of beta thalassemia trait (mean 4.8 million/cumm). Like their study, in our study also MCV and MCH were low in thalassemia. The beta thalassemia major had mean MCV of 70.0 fL and mean MCH of 23.8pg. The beta Thalassemia trait had mean MCV of 65.0 fL and mean MCH of 20.8 pg. However, the lowest level of MCV and MCH was detected for HbE beta thalassemia.

Vehapoglu A et al compared different mathematical indices and found that MCV and RBC counts and their related indices (Mentzer indices etc.), have good discrimination ability in diagnosing beta thalassemia trait.In Mentezer index, if the quotient of the mean corpuscular volume (MCV, in fL) divided by the red blood cell count (RBC, in Millions per microliter) is less than 13, thalassemia is said to be more likely. If the result is greater than 13, then iron-deficiency anemia is said to be more likely. In a lot of cases, the index may fall in between 11 and 13, such cases a peripheral blood smear and iron studies would help to differentiate iron deficiency from thalassemia [13].

HbA2 analysis is considered the gold standard for diagnosing thalassemia. But some cases of thalassemia carriers can have normal HbA2 levels. Presence of microcytosis with HbA2 and HbF concentrations within the reference range made it difficult to interpret. This may be due to iron deficiency or α-thalassemia trait. Several studies have shown that HbA2 synthesis is directly affected by iron deficiency. Iron deficiency anemia produces reduction of MCV, MCH and hemoglobin levels and normal or lowered RBC depending on the severity at the time of hematological analysis. For this reason, iron deficiency anemia can be taken as differential diagnosis for some forms of heterozygous Thalassemia. Raised RBC with low MCV and MCH is more consistent with α thalassemia trait.

In beta thalassemia carriers presenting with a normal HbA2 level, the most frequent cause is co-associated iron deficiency anemia in this study. Moreover, we prefer to perform electrophoresis before any blood transfusion to rule out the possibility of affected hemoglobin pattern in case of multiple transfusion.

CONCLUSION

HbA2 levels are considered to have diagnostic significance in some hemoglobinopathies but due to alteration in HbA2 levels associated with different conditions, it is important to rule out possibility of those conditions to avoid misleading interpretations.

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Nil

CONFLICT OF INTEREST

None

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