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Original Article

Distribution of hemoglobinopathies in patients presenting for electrophoresis and comparison of result with High performance liquid chromatography

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Keywords:

Electrophoresis;
Hemoglobinopathy;
HPLC;
Thalassemia;
Sickle cell.

ABSTRACT

Background: Nearly 226 million carriers of thalassemias and abnormal hemoglobin are present worldwide according to the World Health Organization (WHO). The laboratory plays an important role in the investigation of the thalassemias and hemoglobinopathies. Cellulose acetate electrophoresis at alkaline pH and diagnosis based mainly on visual impression of thickness of band may miss the thalassemic trait patients. The aim of this study was to find out different hemoglobinopathies and thalassemia presenting in our hospital and to compare electrophoresis results with HPLC.

Materials and Methods: This study was performed in the hematopathology section of Department of Pathology of Tribhuvan University Teaching Hospital on cases sent for electrophoresis during 18 months period from October 2013 to March 2015 and included hemoglobinopathies and thalassemias identified by either electrophoresis or HPLC. 97 cases fulfilled the inclusion criteria and thus were included in the study. Electrophoresis at alkaline pH was done in all where as HPLC was performed in 27 cases.

Results: A sharp peak of hemoglobinopathies and thalassemias was seen in Tharu community though other communities are also affected. Thalassemia trait was the most common diagnosis (26.8%) followed by sickle cell anemia (21.6%). Electrophoresis was efficient in detecting some alpha thalassemia variants but missed many cases of beta thalassemia trait.

Conclusion: Beta Thalassemia trait and sickle cell anemia both are common in Nepal , along with some other hemoglobinopathies A sharp peak of hemoglobinopathies and thalassemias are seen in Tharu community. These abnormal hemoglobins and thalassemias are mainly seen in Terai region. Electrophoresis fails to quantify hemoglobin percentage and thus is not appropriate test in beta thalassemia screening.

INTRODUCTION

The World Health Organisation (WHO) reports that the frequency of thalassemias and hemoglobinpathies carriers is 5.1% with nearly 226 million carriers worldwide.[1]

For the most severe cases, the only curative treatment is bone marrow transplantation with a human leukocyte

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Table 1: Age distribution in different hemoglobinopathies and thalassemias

	Age group in years										
Diagnosis	0-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	5-10	>40	Total
Thalassemia Trait	2	1	2	-	4	7	3	1	-	6	26
Sickle cell anemia	4	5	1	5	2	2	2	-	-	-	21
Alpha thalassemia	4	2	3	-	-	1	-	-	-	1	11
HbE beta thalassemia	4	3	-	-	-	1	-	-	1	-	9
Thalassemia major	6	1	1	-	-	-	-	-	-	-	8
`Transfused	-	-	-	-	2	2	1	-	-	-	5
HbE trait	1	1	2	1	-	-	-	-	-	-	5
Sickle cell beta thalassemia	-	2	-	1	-	-	-	-	-	1	4
Delta Beta Thalassemia	1	-	-	-	1	-	-	-	-	1	3
Sickle cell trait	-	1	-	-	-	-	1	-	-	-	2
HbM	-	-	-	-	-	1	-	-	-	-	1
HbE homozygous	-	-	-	-	-	1	-	-	-	-	1
HbD	-	-	-	-	-	-	-	-	-	1	1
Total	**22**	**16**	**9**	**7**	**9**	**15**	**7**	**1**	**1**	**10**	**97**

Table 2: Sex distribution in different hemoglobinopathies and thalassemias

Diagnosis	Female	Male	Total	Percentage
Thalassemia Trait	12	14	26	26.8
Sickle cell anemia	5	16	21	21.6
Alpha thalassemia	1	10	11	11.3
HbE beta thalassemia	2	7	9	9.3
Thalassemia major	5	3	8	8.2
Transfused	2	3	5	5.2
HbE trait	3	2	5	5.2
Sickle cell beta thalassemia	1	3	4	4.1
Delta Beta Thalassemia	1	2	3	3.1
Sickle cell trait	1	1	2	2.1
HbM	0	1	1	1
HbE homozygous	0	1	1	1
HbD	0	1	1	1
Total	**33**	**64**	**97**	**100**

antigen (HLA) compatible sibling. For the majority of patients, therefore, treatment remains supportive and consists of lifelong transfusion/chelation and management of acute and progressive organ damage.[2] Thus management of these diseases pose a significant burden on the healthcare system and family.

In several countries; there are screening programs with the aim of identifying carriers of hemoglobin disorders in order to assess the risk of a couple having a severely affected child and to provide information on the options available to avoid such an eventuality. Nepal doesn't have any such programme and there has been no population based study to find out the prevalence of thalassemia and hemoglobinopathy in Nepal. However there are some published data about such disorders in Nepalese population.[3,4]

The aim of this study was to find out different hemoglobinopathies and thalassemia presenting in our hospital and to compare electrophoresis results with HPLC.

MATERIAL AND METHODS

This study was performed in the Hematopathology section of Department of Pathology of Tribhuvan University Teaching Hospital (TUTH) on cases sent for electrophoresis, during 18 months period from October 2013 to March 2015 with the aim to identify differerent types of hemoglobinopathies and thalassemias presenting to TUTH, the ethnicity and hemogram findings of such patients and to compares electrophoresis and high performance liquid chromatography results. All electrophoresis performed in the department during the study period was evaluated. Before electrophoresis was performed, a complete blood count (CBC) by automated hematology analyser and peripheral smear examination was performed on all cases. Our laboratory only has facility of cellulose acetate electrophoresis at alkaline pH and our diagnosis is based mainly on visual impression of thickness of band seen. Some cases where hemogram findings were suspicious of thalassemia trait but electrophoresis did not show prominent band at A2 position were also sent for high performance liquid chromatography (HPLC). Some other cases were also randomly selected and sent for HPLC at

Table 3: Mean hemoglobin and red cell parameters in different hemoglobin variants

Diagnosis	Hb (gm/dl)	RBC (millions /cu mm)	MCV (fl)	MCH (pg)	RDW
Thalassemia major	4.4	2.4	69.7	21.6	33.7
HbE beta thalassemia	6.0	3.3	60.8	17.7	30.7
HbE homozygous	7.1	4.4	58.0	16.0	27.0
Transfused	7.5	3.1	71.9	26.4	25.5
sickle cell anemia	7.9	3.4	74.7	22.8	22.7
Sickle cell trait	8.3	3.6	70.7	24.1	16.5
HbH	8.3	4.7	64.4	23.6	23.7
Sickle cell beta thalassemia	8.4	4.2	62.0	21.7	18.4
Delta Beta thalassemia	9.2	4.7	70.0	22.5	18.1
Thalassemia Trait	10.6	5.2	65.5	20.3	17.9
HbJ	10.7	3.6	93.2	30.1	15.2
HbE trait	12.9	5.1	77.5	25.0	14.2
HbD	14.5	4.9	82.3	29.9	13.8
HbM	**20.7**	**6.0**	**112.2**	**34.6**	**15.9**

the cost of investigator. Screening of parents was advised in all cases though it could not be done in all cases due to unavailability of one or both parents.

So finally this study included cases with hemoglobinopathy diagnosed by either electrophoresis and/or HPLC. Those cases with suspected hemoglobinopathy but normal electrophoresis or HPLC findings were excluded

Cellulose acetate Electrophoresis at alkaline pH was performed and interpreted according to standard protocol. Hb F was quantified by alkali denaturation method. Sickling test was done where band was seen at HbS position and supravital stain for HbH inclusions were done where a fast band was detected in electrophoresis. Consent was taken from all patients and from parents where patient was a minor. Ethical clearance to perform the study was taken from research ethical committe of TUTH. Data were collected on preformatted questionnaire and result was analysed using SPSS 19 for windows.

RESULTS

Two hundred thirty eight electrophoreses were performed in Department of Pathology from October 2013 to March 2015. Electrophoresis was performed in all cases. 30 were randomly selected for HPLC. This mostly included cases where hemogram and red cell indices were suspicious of thalassemia but electrophoresis was normal. Few other different cases were also included. Out of 30, three were normal in HPLC as well as electrophoresis and thus were excluded from the study. Twenty seven HPLC findings were analyzed. Combining the electrophoresis and HPLC result where available, 97 cases of hemoglobinopathy and thalassemia were identified during the study period and included in study.

There were 64 (66%) males and 33(34%) female. Diagnosis, age group and sex distribution are given in fig 1,table 1 and table 2 respectively. The age of patients ranged from 3 months to 81 years, however most patients were in 0-5 years age group (22.6%)

Thalassemia trait was the most common diagnosis (26.8%) in our study followed by sickle cell disease (21.6%). Out of 11 cases of alpha thalasssemia, 9 were HbH and 2 were Hb J.

In our study address was ascertained in only 35 cases. Commonly effected districts included Bara, Parsa, Bardiya, Dang, Kapilvastu, Kailali and Nawalparasi though occasional cases were also reported in kaski, Kathmandu, Dhanusha and Dhading

The thalassemias and hemoglobinopathies were mainly seen in the Tharu community (37.1%), though wide variety of castes were effected (Fig 2).

Red Cell indices and and red cell distribution width(RDW) were evaluated; in different thalassemias and hemglobinopathies; and are shown in table 3.

Lowest hemoglobin was seen in thalassemia major followed by compound heterozygous for HbE beta thalassemia. RBC count was also lowest for thalassemia major where as beta thalassemia trait had highest RBC count. Compound heterozygous or homozygous state of beta thalassemia, HbH disease and homozygous HbE presented with microcytic hypochromic anemia whereas sickle cell disease and HbE trait had normocytic normochromic or mild microcytic hypochromic blood picture. RDW was highest for thalassemia major and compound heterozygous for HbE beta thalassemia. Correlation between result of electrophoresis

Table 4: Correlation between impression made by electrophoresis and HPLC

HPLC impression	Number	Electrophoresis impression
Thalassemia trait	11	Thalassemia trait (4) Normal band (7)
HbE beta thalassemia	4	HbE beta thalassemia (4)
HbE trait	3	HbE trait (3)
sickle cell anemia	3	Sicke cell anemia (3)
HbH	2	Hb H (2)
HbE homozygous	1	HbE homozygous (1)
J Band	1	Hb J (1)
No opinion. Review after 1 year of age	1	HbE beta thalassemia (1)
Normal	1	Hb M (1)
Total	27	27

Table 5: Mean HbF % in different hemoglobin variants

Diagnosis	Mean HbF	Std. Deviation
Thalassemia major	81.9	9.8
HbE beta thalassemia	26.6	16.9
Transfused	21.0	27.1
sickle cell beta thalassemia	18.3	3.5
Delta Beta Thalassemia	14.0	2.6
sickle cell anemia	9.7	6.6
Hb J	3.6	2.0
HbE homozygous	2.8	0
Sickle cell trait	1.3	0.4
Thalassemia Trait	1.2	0.8
HbE trait	1.1	0.1
HbH	1.0	0.1
HbM	1.0	0
HbD	0.8	0

and HPLC is shown in table 4 Electrophoresis was not able to detect 7 cases of beta thalassemia trait. The figure may be higher because not all such cases were sent for HPLC.

There was an eight month old child with anemia and microcytic hypochromic blood picture who had strong band at HbA2 position and Hb F position, Hb F was 40% , Band at Hb A position was faint .This was considered as compound heterozygous for Hb E -beta thalassemia by electrophoresis as hemogram findings and family study was suggestive. One of the parent was Hb E trait and one was Beta thalassemia trait. But no opinion was made in HPLC, may be considering the age of patient. HbA here was 5.8% and hemoglobin eluting at HbA^2 position was 46.8% in HPLC in this case. Hb J variant showed peak in HPLC however impression of HbH was made by presence of peak before Hb F. HbH can be seen better as fast moving band in electrophoresis and by HbH inclusion in supravital stain. In electrophoresis, in Hb M, strong band was seen and Hb F position but Hb F percentage calculated by alkali denaturation method was only 1%. So a conclusion was drawn that it was a band comigrating with HbF.

Percentage of different hemoglobins detected by HPLC in various conditions are given in table 6.This includes only cases in whom HPLC findings were available

Mean HbF level in different abnormal hemoglobin is shown in table 5. Highest mean HbF was seen in thalassemia major (81.9%) followed by compound heterozygous for HbE beta thalassemia.

DISCUSSION

The prevalence of thalassemias and hemoglobinopathies varies with geographic locations. In Southeast Asia α-thalassaemia, β-thalassaemia, hemoglobin (Hb) E and Hb Constant Spring (CS) are prevalent. Hb E is the hallmark of Southeast Asia attaining a frequency of 50-60 per cent at the junction of Thailand, Laos and Cambodia. Hb CS gene frequencies vary between 1 and 8 per cent.[5]

The disorders of Hb frequently encountered in India include beta thalassemia, HbE - beta thalassemia, HbE, HbD and sickle cell anemia. In study by Mondal et al beta thalassemia trait was the most common abnormality found , followed by HbE trait and then E-beta thalassemia followed by beta thalassemia major/intermedia. Other variants detected included sickle cell trait, HbE disease, sickle cell disease, sickle β thalassemia, HbD-Punjab trait, double heterozygous state of HbS and HbE, double heterozygous state of HbS and HbD, Hb Lepore, HbJ-Meerut and HbH.[6] In study of Goswami et al it was found that Hb E trait was the most common hemoglobinopathies (34.4%) followed by homozygous E (25.3%), beta-thalassemia trait (17.8%), E-β-thalassemia (15.1%), β-thalassemia major (1.5%), sickle cell-β-thalassemia (3.4%), sickle cell trait.[7] Study done by Mehandi et al in Saudi population found Beta Thalassemia trait to be the most common hemoglobinopathy detected followed by Sickle cell trait and sickle cell alpha Thalassemia trait. The Hb variant E and D, which are more prevalent in Southeast Asia were rarely found among Saudis.[8] 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.[9] In our study, beta thalassemia trait was most common, followed sickle cell anemia and then different variants of alpha thaassemia. Other variants detected included compound heterozygous for HbE beta Thalassemia, thalassemia major, sickle cell beta Thalassemia, Hb E trait, and one case each of delta beta thalassemia, HbD and HbM.

HbE occurs at an extremely high frequency in many countries in Asia. Because there is also a high frequency of different beta-thalassemia alleles in these populations, the coinheritance of HbE and beta thalassemia, HbE beta

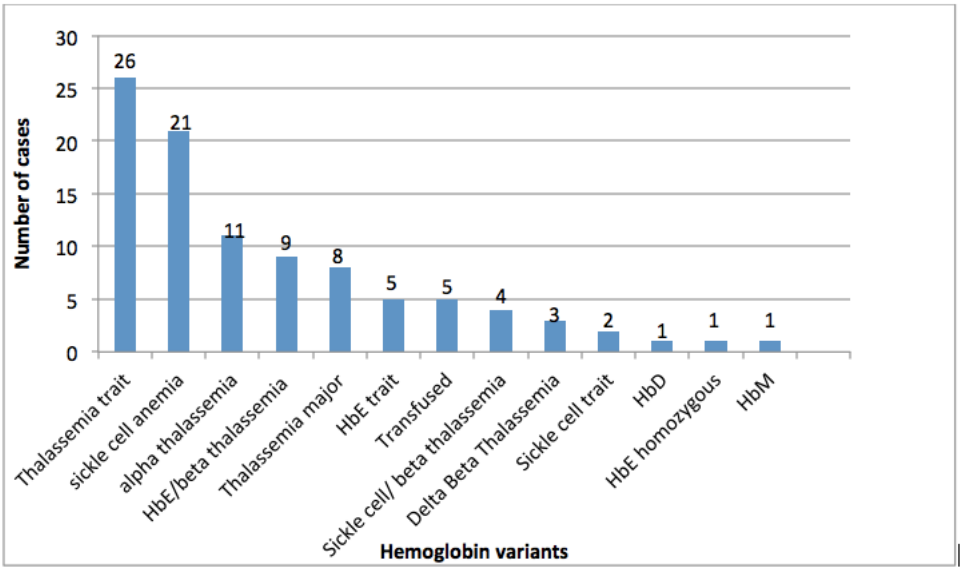

Figure 1: Frequency of different hemoglobinopathies and thalassemias

thalassemia, occurs very frequently.[10] In our study also 9.3% abnormal hemoglobins were HbE beta Thalassemia. Although molecular analysis was not done in these cases, diagnosis was made by combination of electrophoresis findings and by screening of parents. These patients had absent HbA band and increase HbF and HbA2. One parent of these patients 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 selected cases with abnormal electrophoresis findings, this may be the reason of low number of HbE homozygous and HbE trait. Since these patients are asymptomatic may not have presented to hospital or may not have been referred for electrophoresis. The Hb E trait included in our study were also asymptomatic patients, their electrophoresis being run as part of family screening of patients having abnormal electrophoresis.

Fifity three percent were male and 47% were females in study of Manan et al.[11] Similar data was also found earlier by Yagnik and Balgir and reported 65.5, 56 and 62.1% of male patients, respectively.[12,13] 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.[11]

Certain communities in India like Sindhis, Gujratis, Punjabis, and Bengalis are more commonly affected with beta thalassemia, the incidence varying from 1 to 17%. Some population groups from the north eastern regions have a high prevalence of HbE.[14] In study of Goswami et al occurrence of hemoglobinopathies was highest (72.1%) among Rajbanshis followed by Muslims (54.9%). In tribes like "Santal" and "Oraowo" and in Bengali Hindu and Marwari/Behari approximately equal percentage (34%) was observed while least belonged to mongoloid like "Nepalis" and other 'Hill men' population (17.5%).[15] In our study also, maximum number of patients belonged to Tharu community (37.1%). Although abnormal hemoglobins were also found in other variable number of castes, most of them belonged to Terai region. Study done in Nepal on sickle cells anemia by Shrestha A and Karki S also showed that sickle cell anemia was most common in the Tharu community.[5]

In study of Mehdi SR et al, MCV and MCH were significantly low ($P < 0.001$) in cases of thalassemias 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 thalassemias while it was not much affected in sickle cell disorders. The indices were lower in sickle cell α thalassemia trait.[9] 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-thalassemias. However, microcytosis was more marked in β thalassemia trait compared to heterozygous α-thalassemias.[16]

In our study, the mean hemoglobin as well as RBC count was lowest for beta thalassemia major (4.4 gms/dl,), followed by HbE beta thlassemia (6gm/dl). Sickle cell anemia patients had lower mean hemoglobin level than beta thalassemia traits (7.9 gm/dl vs 10.6 gm/dl). While RBC count was normal in sickle cell anemia (mean 3.4 milliom/cumm),it was elevated in case of beta thalassemia trait(mean 5.2 milliom/cumm). Like their study, in our study also MCV and MCH were low in thalassemia. The beta thalassemia

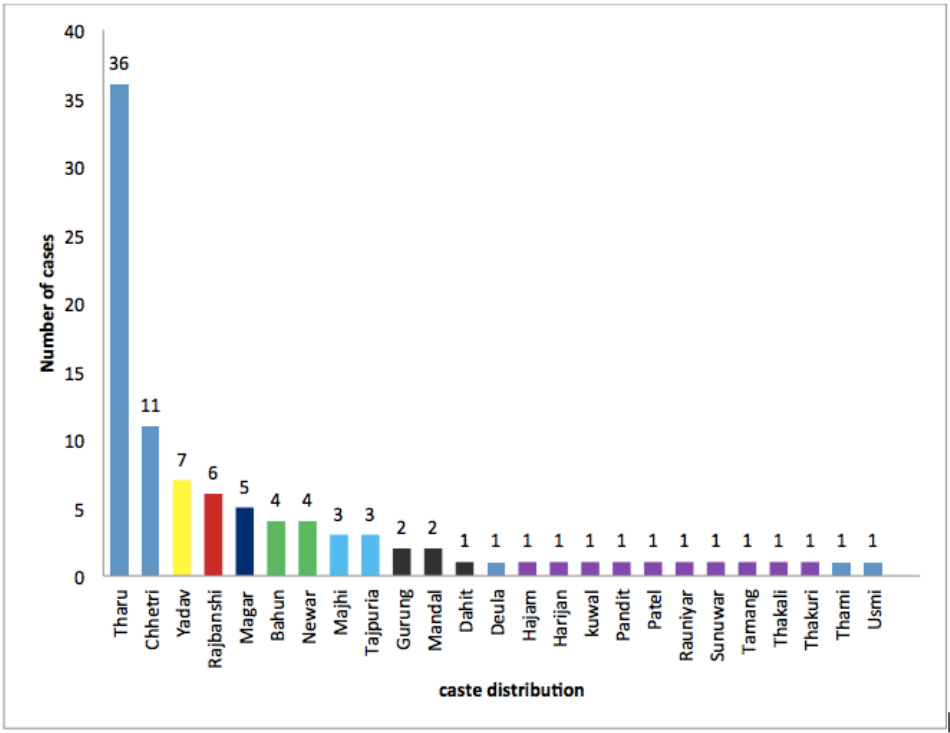

Figure 2: Caste distribution of cases

major had MCV in range of 64.3 to 78.5 fl (mean 78.5) and MCH in range of 18.9 to 26 pg (mean 21.6). The beta Thalassemia trait had MCV in range of 56 to 75.4 fl (mean 65.4) and MCH in range of 16.5 to 23.1pg (mean 20.3). However the lowest level of MCV and MCH was detected for HbE disease.

Many investigators have used different mathematical indices to distinguish beta thalassemia trait from iron deficiency anemia, using only a complete blood count. This process helps to select appropriate individuals for a more detailed examination; however, no study has found 100% specificity or sensitivity for any of these RBC indices. Vehapoglu A et al compared different mathematical indices and found that MCV and RBC counts and their related indices (Mentzer index and Ehsani index), have good discrimination ability in diagnosing beta thalassemia trait.[17] 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 microlitre) 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.[18] However another study suggests Srivastava formula to be more reliable.[19]

The HbA2 analysis is considered the gold standard for diagnosing thalassemia. Several studies have shown that iron deficiency directly affects the rates of HbA2 synthesis in bone marrow; therefore, 16–20 weeks of iron therapy should be instituted, after which a repeat serum iron with electrophoresis should be done to confirm improvement in the HbA2 levels.[20] The most common problem is the presence of microcytosis with HbA2 and HbF concentrations within the reference range. This may be due to iron deficiency or α-thalassemia trait. Iron deficiency anemia produces a wide range of red cell abnormalities (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 easily mistaken for some forms of heterozygous Thalassemia. Raised RBC with low MCV and MCH is more consistent with α thalassemia trait.[21] The Hb A2 level may be modified by many factors. The most frequent problem is the co-existence of an iron deficiency which may even normalize the Hb A2 level requiring a novel Hb assay after iron deficiency treatment. In beta thalassemia carriers presenting with a normal Hb A2 level, the most frequent cause is a co-inherited delta globin abnormality. Increased levels of Hb A2 may result from the co-existence of a variant with electrophoretic or chromatographic properties close to that of Hb A2. As a rule, this situation has to be verified when a level of Hb A2 higher than 8 per cent is observed. It is always better to perform hemoglobin electrophoresis before any blood transfusion because though single transfusion does not affect hemoglobin pattern in electrophoresis but multiple transfusion shows significant difference. Quantitation of HbF is more important than HbA2 in beta thalassemia major. A2 percentage is normal in Thalassemia major due to

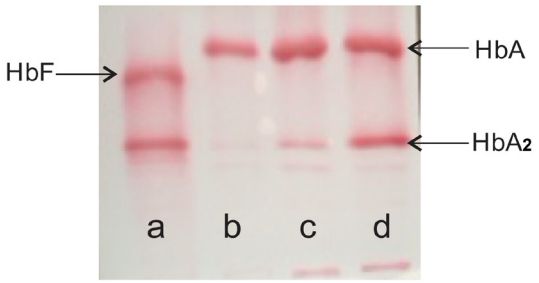

Figure3: a)E beta thalssemia showing strong band at HbF abd HbA2 position
b)normal control showing strong band at HbA position
c)Beta thalassemia trait showing strong band at HbA and visible band at HbA2 position which is stronger than that of normal control
d) Hb E trait showing strong band at Hb A and HbA2 position

increased number of HbF cells which have decreased HbA2 content. In our study the mean HbA2 in beta thalassemia trait was 5.1% (range 4.7 to 5.5) whereas mean HbA2 was 4.3 %in cases of sickle cell beta thalassemia.

Intermittently transfused β-thalassemic major patients showed both 'A' and 'F' band thicker and prominent, A band being thicker than F band in most cases. Whereas regularly transfused patients showed A band mainly, HbF band seen only in few cases in study by Paunipagar et al.[22] Six patients in our study showed strong A and F band and no other band. Two of these were delta beta thalassemia who were asymptomatic and had never received transfusion whereas the other four were transfused Thalassemia major.

The highest adult levels of HbF are seen in beta and delta beta thalassemia, or hereditary persistence of fetal hemoglobin (HPFH), in which HbF can constitute up to 100% of the hemoglobin. In sickle cell disease, HbF usually constitutes only between 5% and 20% of the total hemoglobin. In the presence of some hemoglobin variants (HbS, HbC, Hb Lepore and some unstable hemoglobins) and in association with the beta -thalassemia trait, a slight increase (1 to 5%) of HbF is found in the heterozygotes, while higher levels can be found in the homozygous. HbF levels are variable (10-80%) in presence of HbE and beta thalassemia, the important determinants being the age, the presence of alpha-thalassemia and of genetic determinants of gamma chain synthesis. Increase in HbF keeps HbS more soluble in the deoxygenated state, and the illness is thus less severe.[23] In our study highest level of HbF was found in beta Thalassemia major (mean 84.9%, range 64% to 95%). This was followed by compound heterozygous for HbE /beta thalassemia (10-55%). Sickle cell anemia in our study had HbF upto 25% (mean 9.6%). Average Hb F was 19.62% in sickle cell disease in study of Shrikhande AV et al .Increase need for erythropoiesis because of chronic hemolysis or hematuria and pregnancy can precipitate Vitamin B12 and folic acid deficiency in Sickle cell disease leading to macrocytosis.[24] However macrocytosis was not seen in any sickle cell anemia patients in our study. The MCV in sickle cell anemia ranged from 63 to 89 fl in our study(mean 74.7 fl)

Hemoglobin levels in HbE beta thalassemia range widely between the different phenotypes, from 3 g/dl or less to as high as 11 g/dl. Mean level of HbF can range from 10-50%. 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 βbeta thalassemia.[25] In our study hemoglobin in HbE beta thalassemia ranged from 4.3 to 7.9gm/dl. Mean HbF in these cases was 30.9% (range 16% to 42.8%)in our study. The red cell indices were normal to slightly microcytic hypochromic and Red cell distribution width (RDW) ranged from 12.4 t0 16.5 (Mean 14.1). HbF was also not elevated. There was only one case of HbE homozygous in our study. This patient had hemoglobin 7.1 gm/dl but showed marked anisocytosis and low MCV and MCH. This patient's RDW was 27.

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 < 100 g/ could be used for screening of beta-thalassemia/HbE disease.[26] Mean HbF in HbE beta Thalassemia cases in our study was 26% (range 5-55%). These patients had hemoglobin ranging from 4.1 to 7.9 gm/dl (mean 5.9 gm/dl)and MCV ranging from 55.2 to 64 fl(mean 60.8 fl). 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 beta thalassemia trait.

History of recent blood transfusion must be sought along with correct age so as to aid in an accurate diagnosis. Conditions with borderline Hb A2 need careful interpretation. Iron deficiency may lead to low Hb A2 and hence may mask a thalassemia trait whereas B12/folate deficiency may lead to slightly raised Hb A2 leading to a false diagnosis of a trait. [27]

Hemoglobin electrophoresis is a labor intensive and time consuming method and is not that efficient when quantifying low concentrations of HbA2 and HbF. The HPLC method is a sensitive and precise method and has become the preferred method for thalassemia screening because of its simplicity, superior resolution, rapid assay time and accurate quantification of Hb fraction.[28]

CONCLUSION

Beta Thalassemia trait and sickle cell anemia both are common in Nepal , along with some other hemoglobinopathies A sharp peak of hemoglobinopathies and thalassemias are seen in Tharu community though other communities are also effected. These abnormal hemoglobins and thalassemias are mainly seen in Terai region. Lowest hemoglobin was seen in thalassemia major followed by compound heterozygous for HbE beta thalassemia. Electrophoresis is time consuming and labour intensive method that fails to quantify hemoglobin percentage and thus is not appropriate test in beta thalassemia screening which is diagnosed by elevated HA2 level. As identification of traits is necessary to reduce the birth of thalassemia major cases, it should be used only in conjuction with more advanced techniques like HPLC or others. This study is only hospital based and diagnosis is mainly based on combination of different findings and not on genetic analysis. This study provides only a glimpse of different abnormal hemoglobins and their ethnic distribution. To know the exact burden of thalassemias and hemoglobinopathies and their ethnic and geographic distribution, community based studies are required and molecular methods should be used for mutation identification

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REFERENCES

1. WHO Guidelines for the control of hemoglobin disorders. In: Model B (ed). World Health Organization Hereditary Disease Programme. Document of the VIth annual meeting of WHO working group on hemoglobinopathies, Sardinia, 1989; 1994.1–6

2. Bouva MJ, Mohrmann K, Brinkman HB et al. Implementing neonatal screening for haemoglobinopathies in the Netherlands. J Med Screen 2010;17:58-65. Crossref

3. Sakai Y, Kobayashi S, Shibata H, Furuumi H, Endo T, Fucharoen S, Hamano S, Acharya GP, Kawasaki T, Fukumaki Y. Molecular analysis of alpha-thalassemia in Nepal: correlation with malaria endemicity. J Hum Genet 2000;45:127-32. Crossref

4. Mishra A, Mukherjee A, Roy A et al. Distribution and ethnic variation of beta-thalassemia mutations in Nepal. Nepal Med Coll J 2012;14:49-52. Crossref

5. Shrestha A, Karki S. Analysis of sickle hemoglobin. Journal of Pathology of Nepal 2013;3:437-40. Crossref

6. Suthat Fucharoen S, Winichagoon P. Haemoglobinopathies in Southeast Asia. Indian J Med Res 2011;134:498–506. PMid:22089614 PMCid:PMC3237250

7. Mondal SK, Dasgupta S , Mondal S, Das N. Spectrum of thalassemias and hemoglobinopathies in West Bengal: A study of 90,210 cases by cation exchange high-performance liquid chromatography method over a period of 8 years. Journal of applied Hematology 2014;5:91-5. Crossref

8. Goswami BK, Pramanik R, Chakrabarty S, Pal PP, Banerjee S, Bandyopadhyay A. Spectrum of hemoglobin variants in the population of northern region of West Bengal: An ethnogenetic proposition. J Family Med Prim Care 2014;3:219-23. Crossref

9. Mehdi SR, Al Dahmash BA. Analysis of hemoglobin electrophoresis results and physicians investigative practices in Saudi Arabia. Indian J Hum Genet 2013;19:337-41Crossref

10. Patel U, Shrivastav A, Joshi J R, Agnihotri A S, Kaur A, Thakkar B. Detection of hemoglobinopathies and thalas- semias in population of Gujarat state using HPLC: Analysis of 2022 cases.Pathology and laboratory medicine 2012;4:80-4.

11. Fucharoen s, Weatherall DJ. The hemoglobin E thalassemias. Cold Spring Harb Perspect Med 2012;2:a01173.

12. Mannan A, Kawser J, Ahmed AMA, Sikder MOF, Islam MJ, Chowdhury MA. A Demographic Approach for Understanding the Prevalence of β Thalassemia Patterns and Other Hemoglobinopathies: Selective Study in Chittagong City Perspective. Asian Journal of Biological Sciences, 6: 124-130.

13. Yagnik H. Post counselling follow-up of thalassemia in high risk communities. Indian Paediatr 1997;34:1115-58. Crossref

14. Balgir R.S.Genetic epidemiology of the three predominant abnormal hemoglobins in India. J. Assoc. Physicians India 1996; 44:25-8. Crossref

15. Colah R, Gorakshakar A, Phansagaonkar S et al. Epidemiology of β-thalassemia is western India: mapping the frequencies and mutation in sub-regions of Maharashtra and Gujarat. Br J Haematol 2010;149:739–47. Crossref

16. Goswami BK, Pramanik R, Chakrabarty S, Pal PP, Banerjee S, Bandyopadhyay A. Spectrum of hemoglobin variants in the population of northern region of West Bengal: An ethnogenetic proposition. J Family Med Prim Care 2014;3:219-23. Crossref

17. Mehdi SR, Al Dahmash BA. A comparative study of hematological parameters of α and β thalassemias in a high prevalence zone: Saudi Arabia. Indian J Hum Genet. 2011;17:207–11. Crossref

18. Vehapoglu A, Ozgurhan G, Dogan Demir AG,et al. Hematological Indices for Differential Diagnosis of Beta Thalassemia Trait and Iron Deficiency Anemia. Hidawi. 2014 [Internet] Available at http://www.hindawi.com/journals/anemia/2014/576738/. Crossref

19. Mazza JJ . Manual of clinical hematology 3rd edition. Philadelphia, Pennsylvania: Lippincott Williams & Wilkins;2002 .2190p.

20. Pornprasert S, Panya A, Punyamung M, Yanola J, Kongpan C. Red Cell Indices and Formulas Used in Differentiation of b-ThalassemiaTrait from Iron Deficiency in Thai School Children. Hemoglobin 2014;38:258–61. Crossref

21. El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anemia on the levels of hemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol 2002;24:285-9. Crossref

22. Dogaru M, Coriu D, Higgins T. Comparison of two analytical methods (electrophoresis and HPLC) to detect thalassemias and hemoglobinopathies. Revista Română de Medicină de Laborator. 2007;9:39–48.

23. Paunipagar PV, Vaidya SM, Singh CM. Changing pattern of Hb electrophoresis and HbA2 levels in β thalassemia major . Indian J. Prev. Soc. Med 2010;4:148-51.

24. Mosca A, Paleari R, Leone D, Ivaldi G. The relevance of hemoglobin F measurement in the diagnosis of thalassemias and related hemoglobinopathies. Clin Biochem 2009;42:1797-801. Crossref

25. Shrikhande AV, Dani AA, Tijare JR, Agrawal AK. Hematological profile of sickle cell disease in central India. Indian J. Hematol. Blood Transfus 2007; 23:92–8. Crossref

26. Fucharoen S, Weatherall DJ. The hemoglobin E thalassemias. Cold Spring Harb Perspect Med2012: 2: a011734. Crossref

27. Pornprasert S, Moriyama A, Kongthai K et al. Detection of beta-thalassemia/hemoglobin E disease in samples which initially were diagnosed as homozygous hemoglobin E. Clin Lab 2013;59:693-7 Crossref

28. Prasad C, Singh SB, Mahato T, Prakash S, Chandra S. An Assessment of the Genetic Load of Hemoglobinopathies by Using Retention Time Chromatogram: A Tertiary Care Hospital Based Study, Located In Ranchi District of Jharkhand. Res J Pharm Biol Chem Sci 2015;51).1088-101.