ISSN: 0975-3583,0976-2833 VOL13,ISSUE07,2022

"Hemoglobinopathies Pattern among High Suspicion Anaemic Patients Attending Tertiary Care Hospital in Telangana"

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ABSTRACT

Background: Hemoglobinopathies are common genetic disorders of haemoglobin, which can be prevented by population screening and offering genetic counseling. The study was aimed to assess the electrophoretic pattern of structural hemoglobin variants and thalassemias among high suspicion cases of anemia.

Material & Method: This cross-sectional study was conducted in clinical biochemistry laboratory of Gandhi Medical College and Hospital during the period of September 2011 – July 2013. The anaemic cases admitted in the inpatient wards of paediatrics, obstetrics and gynaecology, medicine, surgery, orthopaedics based on clinical suspicion, family history, peripheral blood picture, blood indices were screened..

Result: In the present study analysis of data showed out of 123 cases, 39 cases are positive for hemoglobinopathies. 2 cases were inconclusive. Prevalence rate in the present study is 32.2 % Highest positivity rate for sickle cell disease 38.46% has been reported followed by β^0 thalassemia major 17.95%, sickle β thalassemia 15.38%. On analyzing the sex ratio it was observed that 28 % of males and 34.2 % of females were positive for hemoglobinopathies and thalassemias. Female preponderance has been documented. Highest incidence has been reported in the age group 0-15yrs.

Conclusion: A hospital based cross-sectional study showed significant prevalence rate of 32.2% of hemoglobinopathies and thalassemias. Being hospital based study the prevalence of β thalassemia trait and sickle cell trait was underestimated as they are clinically silent and

ISSN: 0975-3583,0976-2833 VOL13,ISSUE07,2022

present to the hospital in rare scenarios therefore population based study will definitely show high prevalence of β thalassemia trait and sickle cell trait.

Keywords: Hemoglobinopathy, Thalassemia, Sickle Cell Disease, Electrophoresis, Consanguinity

Introduction

Hemoglobin is a intracellular protein that gives RBC their color, is one of the best characterized proteins and was one of the first proteins to be associated with a specific physiological function of O_2 transport,[1] efficiently carrying O_2 from lung to tissues and transport of CO_2 and H^+ ions back to lungs.[2,3] The efficiency with which Hb binds and releases O_2 is reminiscent of specificity and efficiency of metabolic enzymes. Because many of the theories formulated to explain O_2 binding to Hb also explain the control of the enzyme activity Hb has been dubbed as Honorary Enzyme.[1]

Hemoglobinopathies are disorders affecting the structure, function, or production of hemoglobin. These conditions are usually inherited and range in severity from asymptomatic laboratory abnormalities to death in utero. Different forms may present as hemolytic anemia, erythrocytosis, cyanosis, or vaso-occlusive stigmata.[4,5] Currently more than 900 hemoglobinopathies have been described, but only 9 have clinical significance.[6,7] Not all Hb variants produce clinical symptoms, but some abnormal Hb molecules do cause debilitating diseases. Naturally occurring Hb variants that are lethal are of course never observed.[1,8] Present study aimed to assess the electrophoretic pattern of structural hemoglobin variants and thalassemias among high suspicion cases presenting with anemia.

Material & Method

This cross-sectional study was conducted in clinical biochemistry laboratory of Gandhi Medical College and Hospital (GMH) during the period of September 2011 –July 2013. The anaemic cases admitted in the inpatient wards of paediatrics, obstetrics and gynaecology, medicine, surgery, orthopaedics based on clinical suspicion, ethnicity, family history, peripheral blood picture, blood indices were screened. Patients clinical history, family history were noted down. Under all aseptic precautions, 5 ml of blood was collected in EDTA vaccutainer and haemogram with reticulocyte count, sickling test, estimation of HbF and electrophoresis were performed. For electrophoresis the samples were stored at 4-8^{o}C and were analyzed in batches within one week.

Red blood cell indices were obtained from cell counter- ABX MICROS 60 hematology analyzer. The key to successful detection of hemoglobinopathies, particularly the thalassemia is the initial hematological data. Low Mean Corpuscular Volume (MCV), low Mean Corpuscular Hemoglobin (MCH) points towards Thalassemia. Blood smear done by Leishman staining were studied to look for ancillary findings like anisopoikilocytosis, polychromasia, target cells, sickle cells and nucleated RBCs.

Special hematological tests:

1. Sickling Test.
2. Electrophoresis:

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Sickling Test Metabisulfite Slide Test.

Principle: Adding sodium metabisulfite, a reducing substance, to blood enhances deoxygenation of Hb and sickling of HbS. The test does not distinguish sickle cell anemia from sickle trait or other HbS syndromes because all red cells sickle. False-negative tests may occur if HbS concentration is less than 10% (as in very young infants), or if deoxygenation is inadequate (e.g., deterioration of reagent).[9]

Electrophoresis: Helena SAS - MX Alkaline Hb-10 kits was used. Both human and $ASFA_2$ Helena controls were run in every batch.

The SAS-MX Alkaline Hb-10 kit is intended for the separation of human haemoglobins by agarose gel electrophoresis. Electrophoresis is generally considered the best method for separating and identifying haemoglobinopathies.[10–16] This method is based on the complex interactions of the hemoglobin with an alkaline electrophoretic buffer and the agarose support. The SAS-MX Alkaline Hb-10 procedure is a simple procedure requiring minute quantities of haemolysate to provide complementary evidence (along with the results from SAS-MX Acid Hb-10 analysis) of the presence of HbS, HbC and HbF as well as several other abnormal hemoglobins.

Sample collection and preparation: Freshly collected EDTA or heparin anticoagulated blood is the specimen of choice. Samples was stored and refrigerated at 2-6°C for up to 1 week. For optimal results, saline washed red cells was used to prepare lysates. This removes possible interference from plasma proteins.

a) Mix 200µL of well mixed whole blood with 1000µL of saline solution.
b) Centrifuge to sediment the red cells.
c) Remove 1000µL of the supernatant and discard.
d) Add a further 1000µL of saline solution and mix well.
e) Repeat steps b-d x2.
f) Following the final centrifugation, remove 1000µL of supernatant and treat the remaining sample as whole blood, or remove all of the supernatant and treat the remaining sample as washed packed cells.

Each patient sample / control was diluted to a hemoglobin concentration of 1.0-2.0 g/dL with Haemoglobin Lysing Agent.

Step-By-Step Procedure:

1. The gel was removed from the packaging and placed on a paper towel. Blot the gel surface with a blotter C, discard the blotter.
2. The sample application template with the arrows at the edge of the gel was aligned. A blotter A was placed on top of the template and a finger was rubbed across the slits to ensure good contact. The blotter was removed and was retained for use in step 5.
3. 3µl of sample was applied to each slit and was allowed to absorb for 5 minutes.
1. Whilst samples are absorbing, 30ml of buffer was poured into each inner section of the SAS-MX Chamber.
2. Following sample absorption, the template was blotted with the blotter A retained from step 2 and remove both blotter and template.

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3. The gel was positioned in the chamber agarose side down, aligning the positive (+) and negative (-) sides with the corresponding positions on the chamber.
4. Electrophorese the gel: 150 volts, 30 minutes
5. At the end of electrophoresis, the gel was dried at 60-70°C.
6. **NOTE:** The drying of the gel should take no more than 5 minutes to prevent diffusion of bands. If this cannot be achieved, fix the gel in fixative solution for 5 minutes prior to drying.
7. The dry gel was immersed in stain solution for 10 minutes.
8. The dry gel was destained in 2 x 1 minute washes of destain solution or until the background is clear.
9. The gel was dried.

INTERPRETATION OF RESULTS

Qualitative Evaluation:

The possible identity of the hemoglobin types present in the samples was determined by visual evaluation of the completed gel. The Hemo controls provide a marker for band identification.

Quantitative Evaluation:

The relative percent of each hemoglobin type on the gel was determined by densitometry of the completed gel at 595nm.

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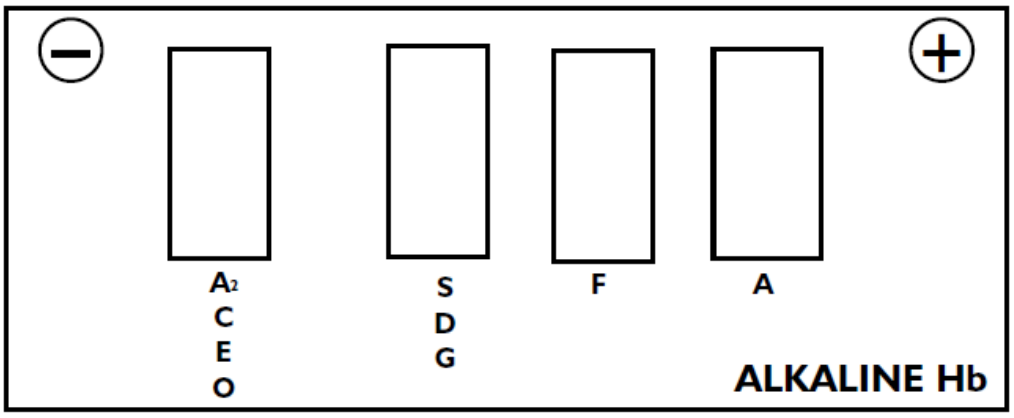

Figure 1: Electrophoretic pattern of common haemoglobin variants

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The most common haemoglobin abnormalities:

Sickle Cell Trait: This is a heterozygous state showing HbA and HbS and a normal amount of HbA_2.

Sickle Cell Anaemia: This is a homozygous state showing almost exclusively HbS, although a small amount of HbF may also be present.

Sickle-C Disease: This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Thalassemia Disease: This condition shows HbA, HbF, HbS, and HbA_2.

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In Sickle Cell β^o-Thalassaemia HbA is absent.
In Sickle Cell β^+-Thalassaemia HbA is present in reduced quantities.
Thalassemia-C Disease: This condition shows HbA, HbF and HbC.
HbC Disease: This is a homozygous state showing almost exclusively HbC.
Thalassaemia Major: This condition shows HbF, HbA and HbA_2.
Further testing required:

1. Acid Hb electrophoresis may be a necessary follow up test for confirmation of abnormal hemoglobins detected.
2. Globin chain analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the more rare hemoglobins.

Reference values: At birth, the majority of hemoglobin in the erythrocytes of the normal individual is fetal hemoglobin, HbF. Some of the major adult hemoglobin, HbA, and a small amount of HbA_2, are also present. At the end of the first year of life and through adulthood, the major hemoglobin present is HbA with up to 3.7% HbA_2 and less than 2% HbF.

Result:

Diagnosis of hemoglobinopathies and thalassemia was made based on the clinical findings, hematologic parameters, special laboratory tests sickling test, electrophoretic pattern. The values of hematologic parameters were expressed as mean. In the present study analysis of data showed out of 123 cases, 39 cases are positive for hemoglobinopathies. On the basis of the clinical history patients aged 2 days M, and 50 days, F was screened for haemoglobinopathies and thalassemias. Electrophoretic pattern showed high percentage of HbF band, as newborn blood contains high concentration of HbF, both the cases were inconclusive, Patient's parents were educated to come for follow up after 1 year. Prevalence rate in the present study is 32.2 %.(39/121).

Table 1: Distribution of demographics of Cases Screened for Haemoglobinopathies and Thalassemias.

		Positive Cases	Negative Cases	Total
Gender	Male	14 (28.57)	35 (71.42)	49
	Female	25 (34.2)	47(65.27)	72
	Total	39 (32.23)	82(67.76)	121
Agewise distribution	0-15 yrs	21	53	74
	16-30 yrs	16	24	40
	31-45yrs	1	3	4
	45-60yrs	1	2	3
	Total	39	82	121

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Consanguinity	Consanguineous	26	22	48
	Non - consanguineous	13	60	73
	Total	39	82	121

On analyzing the sex ratio it was observed that 28.6 %(14/49) of males and 34.2 % (25/72) of females were positive for hemoglobinopathies and Thalassemia. Female preponderance has been documented in the present study. In the present study highest incidence has been reported in the age group 0-15yrs.

Table 2: Category Wise Distribution of Cases of Haemoglobinopathies and Thalassemias

Category	No. of Cases	Percentage
Sickle cell disease	15	38.46
β^0 thalassemia major	7	17.95
Sickle β^0 thalassemia	6	15.38
β thalassemia trait	4	10.256
β thalassemia intermedia	1	2.564
Sickle β^+ thalassemia	1	2.564
Sickle α thalassemia	1	2.564
HbH	1	2.564
Sickle cell trait	1	2.564
HbE -β^0 thalassemia	1	2.564
Inconclusive case	1	2.564

Highest positivity rate for sickle cell disease has been reported followed by β^0 thalassemia major, Sickle β^0 thalassemia. On insistence of pediatrician 14 yr old was screened for sickle Hb after blood transfusion and electrophoretic pattern showed both HbA and HbS Patient was instructed to come after 3 months, patient did not for follow up, so no definitive diagnosis was made

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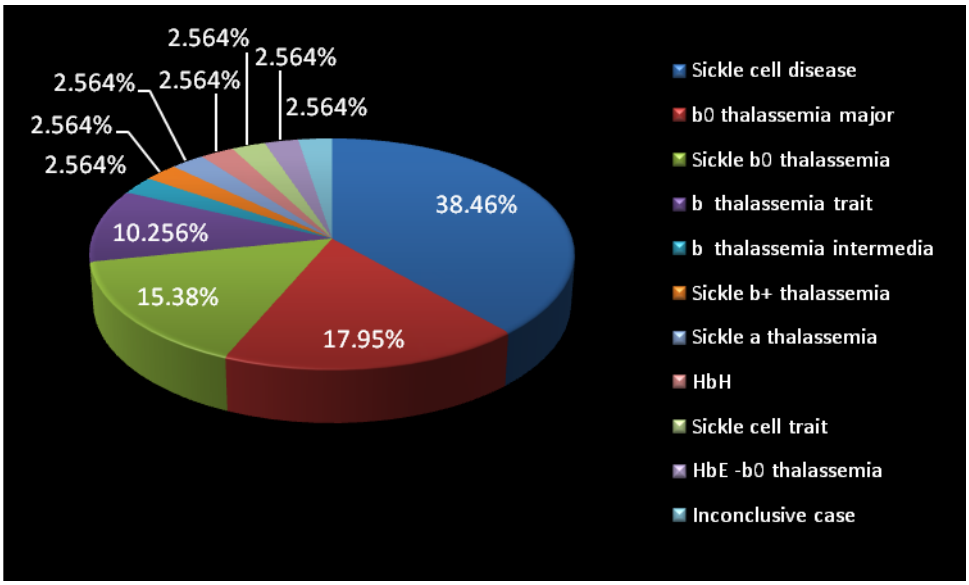

Figure 2: Category Wise Distribution of Cases of Haemoglobinopathies and Thalassemias

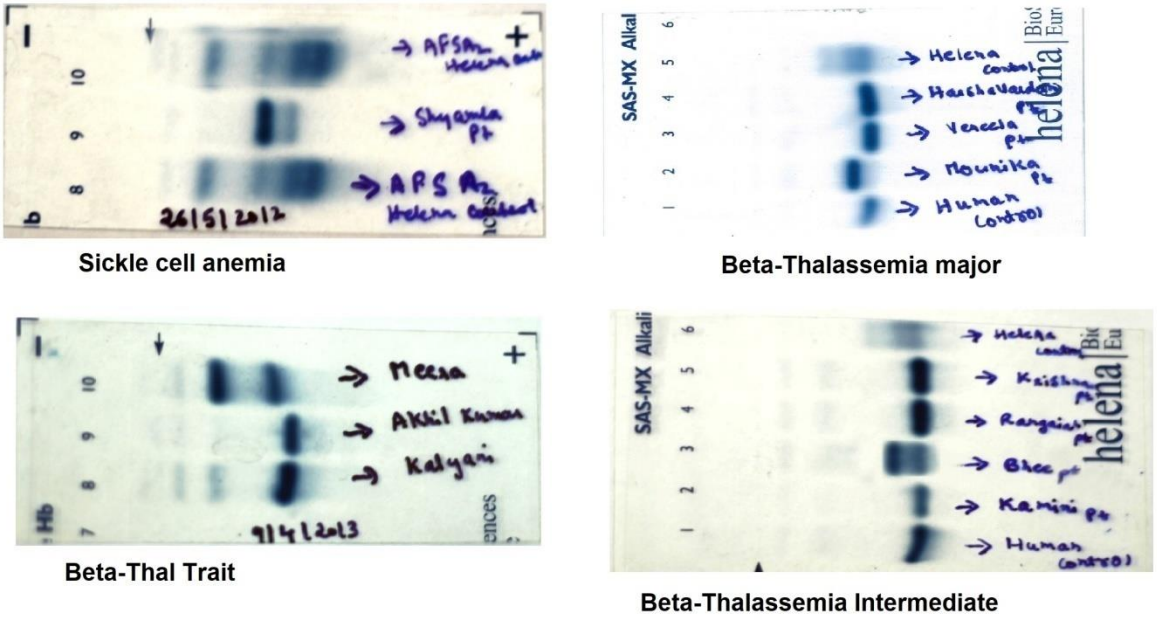

Figure 3: Patterns seen on electrophoresis

Discussion:

India has a population of 1.21 billion according to the Census in 2011[17] and according to the UN report, India's population will reach 1.412 billion in 2022, compared with 1.426 billion in China.[18] There are 4,693 endogamous communities which includes 427 tribal groups. Although β-thalassemia and other haemoglobinopathies are seen in all the states, the prevalence is quite variable.[19]

A total of 123 anaemic cases were screened on the basis of clinical history, hemogram, peripheral blood smear. Amongst the 123 cases screened 39 cases are positive for hemoglobinopathies and thalassemias and 2 cases were inconclusive. On the basis of special

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hematological tests sickling test and electrophoresis, family study positive cases have been categorized into sickle disease, sickle trait, thalassemia major, thalassemia intermedia, thalassemia trait, sickle β^o thalassemia, sickle β^+ thalassemia, sickle α thalassemia, Hb E-β thalassemia and HbH disease.

The present hospital based study using uniform protocols and methodology as well as quality controls showed a prevalence of 31.45% of hemoglobinopathies and β thalassemias. This prevalence rate correlates with other hospital based studies of Jain B.B et al.,[20] which revealed a prevalence rate of 29.3%, Bikash Mond et al.,[21] showed 27.35% prevalence, Shivashankara et al.,[22] revealed 30% positivity for haemoglobinopathies, Seema Rao et al reported 30.9% positivity.[23]

The incidence of hemoglobin variants in our study differs from various prevalent studies done in different geographical areas in India and other countries. The present study had showed high prevalence of sickle cell disease 38.46%. Amrita Panda et al.,[24] Priscilla Chandran et al., also reported high positivity for sickle cell disease.[25]

Jain B.B et al,[20] Bikash Mond et al,[21] Seema Rao et al, Anupam sachdeva et al, Dolai T.K et al reported β thalassemias heterozygous was most frequently seen. Deshpande RH et al,[26] Shah Sejal et al,[27] Shivashankara et al,[22] Giri et al found high posivity rate for β thalassemia major.[28] High prevalence of sickle cell trait was reported by Balgir S et al,[29] Chotray G.P et al.[30] The highest frequency of sickle cell gene in India is reported in Orissa 9% followed by Assam, Madhya Pradesh, Uttar Pradesh, Tamil Nadu and Gujarat.[22] The distribution of β-thalassemia is not uniform in Indian sub-continent. The highest frequency of β-thalassemia trait is reported in Gujarat followed by Sindh, Punjab, Tamil Nadu and Maharashtra.[22]

In this study the incidence of heterozygous state for various Hb variants is less compared to other studies because samples were received from anemic cases who were admitted in inpatient ward. Heterozygous states for various Hb variants are asymptomatic carriers.[31–35]

In India, the problem of hemoglobinopathies is compounded by the heterogeneity of population, the fertility rates, the literacy rates, and the rates of consanguineous marriages are also diverse. Consanguinity in marriages is a well accepted social norm irrespective of religion, caste, educational status and economical background.[22]

Conclusion:

A Hospital based cross-sectional study showed significant prevalence rate of 32.2% of hemoglobinopathies and thalassemias. Being hospital based study the prevalence of β thalassemia trait and sickle cell trait was underestimated as they are clinically silent and present to the hospital in rare scenarios therefore population based study will definitely show high prevalence of β thalassemia trait and sickle cell trait. It is necessary to educate the patients regarding the consequences of consanguineous marriages and the importance of their presentation in the next generation. This study shows the necessity of population based screening to reduce the burden of hemoglobinopathies and thalassemias.

Compliance with Ethical Standards: Authors declare no potential conflict of interest.

Funding: The present study didnot receive any external funding or financial benefits.

Conflict of Interest: Authors declare no conflict of interest.

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Ethical approval: The study obtained the ethics clearance from the institutional ethics committee.

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