Study of thalassemia and other haemoglobinopathies in a reproductive age group by Capillary haemoglobin electrophoresis system in a tertiary care teaching hospital

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ABSTRACT

Context: Haemoglobinopathies are a group of genetic disorders of haemoglobin (Hb). Inherited disorders of haemoglobin synthesis are an important cause of morbidity and mortality worldwide. Population screening, genetic counselling and prenatal diagnosis can prevent these genetic disorders.

Aims: This study aims to screen for thalassemia and abnormal haemoglobin opathies in anaemic patients of reproductive age group for early detection and management and to reduce the rate of affected infants and screening even asymptomatic patients by Capillary haemoglobin electrophoresis and by referring positive cases for genetic counselling.

Methods and Material: This was a hospital based observational study done for a period of three years. Capillary Hemoglobin electrophoresis is a well established technique routinely used in clinical laboratories for screening samples for haemoglobin abnormalities. The MINICAP FLEX-PIERCING instrument has been developed to provide complete automation of this testing with fast separation and good resolution.

Results: In this study, total 100 cases of reproductive age groups were studied. The most common haemoglobinopathy in this study is β thalassemia trait (45%) followed by HbD Punjab

(4%), Sickle cell trait (2%), HbE Beta thal (2%), HbS (1%) and S Beta thal (1%). The inherited disorders of haemoglobin synthesis are one of the important public health problems in India.

Conclusion: The Capillary haemoglobin electrophoresis is an excellent diagnostic tool. It is a well established technique routinely used in clinical laboratories for screening samples for haemoglobin abnormalities. This requires proper health education and adequate sensitization of the individual and community to accept these remedial measures.

Key-words: Haemoglobinopathies, Thalassemia, Reproductive age group, Capillary haemoglobin electrophoresis system.

Key Messages: In view of the frequent requirement of blood transfusions, preventive genetic strategies for haemoglobinopathies and thalassemia are of utmost importance to diagnose these haemolytic disorders in India. Capillary haemoglobin electrophoresis is an excellent diagnostic tool for screening samples for haemoglobin abnormalities.

INTRODUCTION

Haemoglobinopathies are a group of genetic disorders of haemoglobin (Hb).[1]The general incidence of thalassemia trait and sickle cell haemoglobinopathy in India varies between 3-17% and 1-44% respectively[2] but because of consanguinity, caste and area endogamy, some communities show a very high incidence, making the disease a major public health problem in our country.[2,3] Population screening, genetic counselling and prenatal diagnosis can prevent these genetic disorders.

The aim of this study is to screen for abnormal haemoglobinopathies in anaemic patients of reproductive age group for early detection and management and to reduce the rate of affected infants and screening even asymptomatic patients and referring positive cases for genetic counselling.

MATERIALS AND METHODS

This was a hospital based observational study done for a period of three years. One hundred blood samples of reproductive age group cases taken from various departments. A detailed medical history including family genetic history, blood transfusion history and blood related infections history were taken and investigations like complete blood count (CBC), peripheral

blood film (PBF), reticulocyte count, sickling test in relevant cases and capillary haemoglobin electrophoresis were done.

Capillary haemoglobin electrophoresis (CE) is a well established technique routinely used in clinical laboratories for screening samples for haemoglobin abnormalities. The SEBIA MINICAP FLEX-PIERCING instrument has been developed to provide complete automation of this testing with fast separation and good resolution.

It uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated in silica capillaries by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electroosmotic flow.These Hb fractions are directly detected at a specific absorbance of 415 nm.

At the end of the analysis, relative quantification of individual hemoglobin fractions is performed automatically and profiles can be analyzed; the hemoglobin fractions, Hb A, Hb F, Hb A2 are automatically identified.

Normal Values-

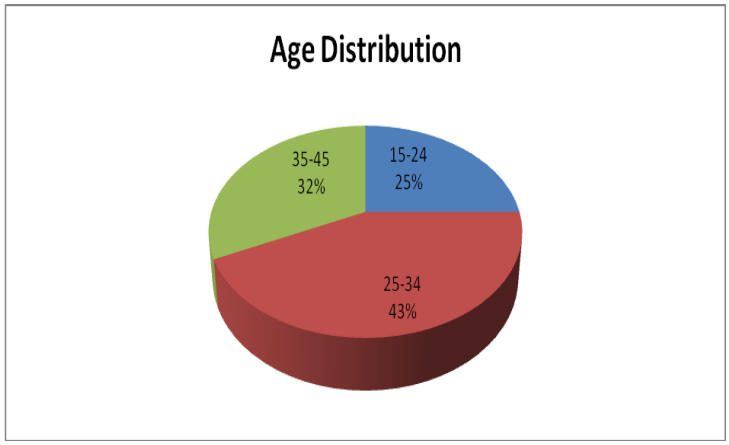
Hemoglobin A: comprised between 96.8 and 97.8 %

Hemoglobin F: < 0.5 %

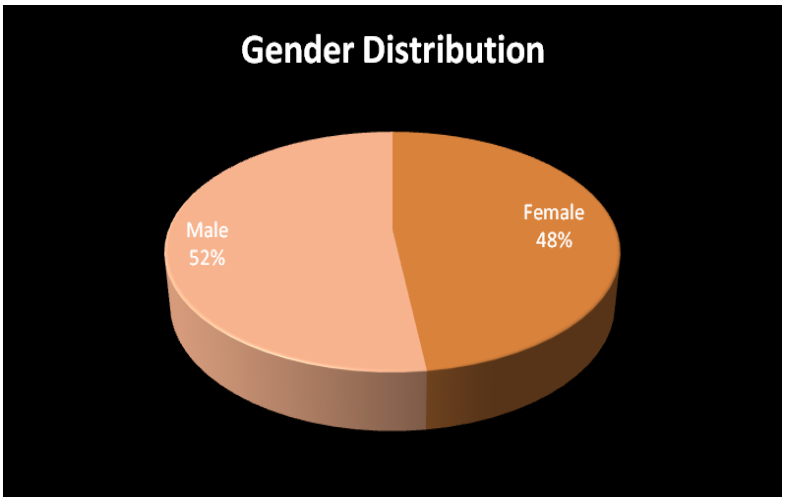
Hemoglobin A2: comprised between 2.2 and 3.2 %

RESULTS

The study was undertaken during a period of three years. Capillary haemoglobin electrophoresis (CE) was performed on one hundred indoor and outdoor reproductive age group patients with signs and symptoms of anemia. A total of 100 cases were studied and the results are put forwarded in tabular, bar diagram & pie chart.



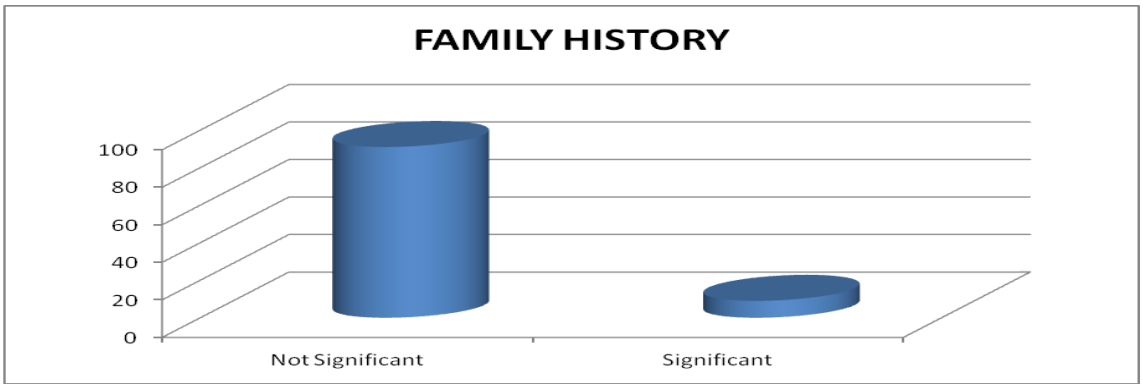
GRAPH 1 shows age distribution of 100 cases screened in which maximum number of cases were in age group of 25-34 years (43%) followed by 35-45 years (32%) and 15-24 years (25%)



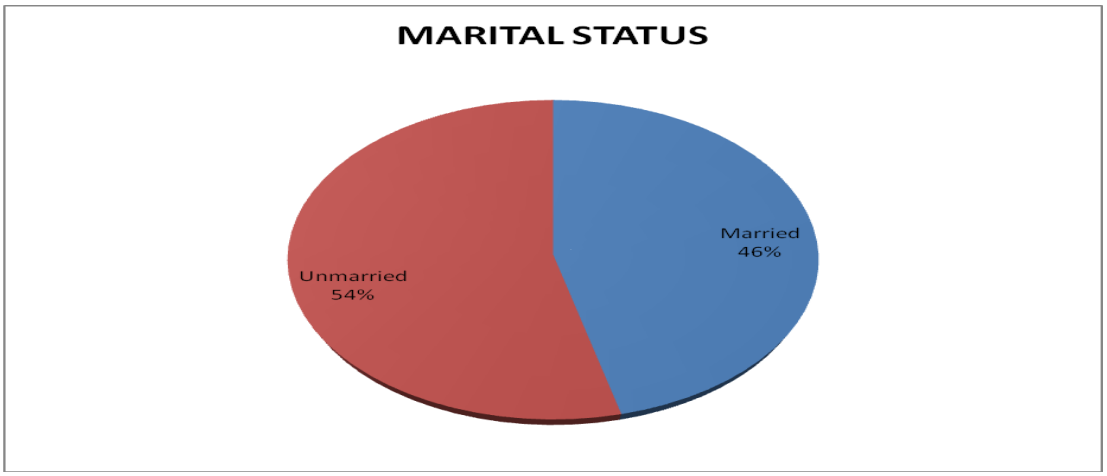
GRAPH 2 - Gender Distribution

RELIGION/COMMUNITY	CE INTERPRETATION							
	Beta Thal Trait	HbD Punjab	HbE Beta Thal	HbS	HbS Trait	SBeta Thal	Normal	Grand Total
Hindu	33	1	1	-	-	1	39	66
Jain	-	-	-	-	-	-	2	2
Muslim	8	-	1	1	2	-	2	14
Punjabi	2	3	-	-	-	-	2	7
Sindhi	2	-	-	-	-	-	-	2
Grand Total	45	4	2	1	2	1	45	100

Table 1shows CE Interpretation according to Religion/Community.



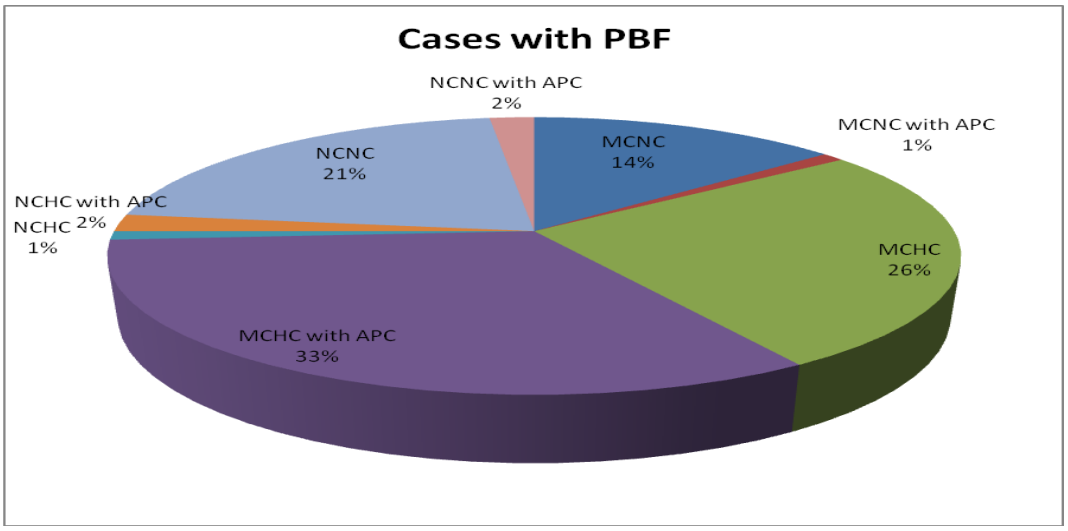
Graph 3 shows distribution according to family history and only 9 % cases have significant family history out of 55 diagnosed cases.



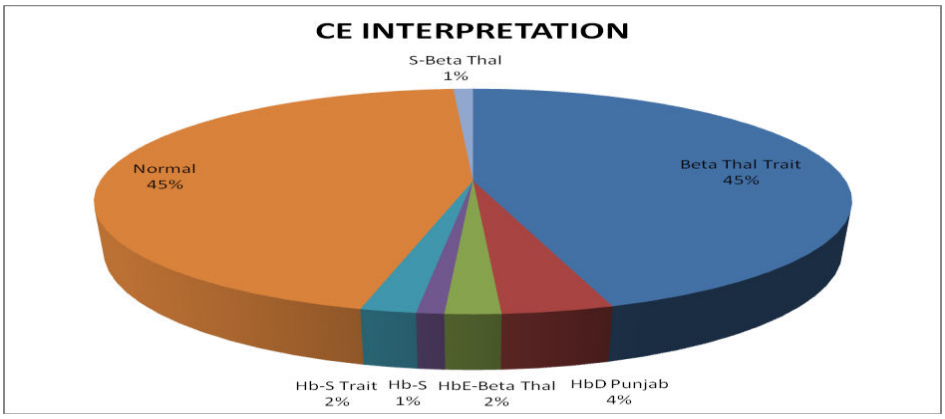
Graph 4 shows distribution according to marital status and 54% cases were unmarried and 46% cases were married.

CHIEF COMPLAINTS	Number of Patients	%
Asymptomatic	6	6
Dyspnoea	1	1
Fatigue	87	87
Pallor	1	1
Pallor with Dyspnoea	5	5
Grand Total	100	100

TABLE 2 - Distribution of patients according to CHIEF COMPLAINTS.



GRAPH 5 - Distribution of cases according to PBF



Graph 6 shows CE Interpretation of total 100 cases in which 45% are normal cases and rest 55% diagnosed cases shows 45% Beta Thal Trait, 4% HbD Punjab, 2% HbE-Beta Thal, 2% HbS Trait, 1% HbS and 1% S-Beta Thal.

Hb (g/dl)	**CE INTERPRETATION**							
	Beta Thal Trait	**HbD Punjab**	**HbE Beta Thal**	**HbS**	**HbS Trait**	**S Beta Thal**	**Normal**	**Grand Total**
<5	-	-	-	1	-	-	7	8
5-10	8	-	2	-	-	-	19	29

10-15	36	2	-	-	1	1	17	57
15-20	1	2	-	-	1	-	2	6
Grand Total	45	4	2	1	2	1	45	100

TABLE 3 - CE Interpretation with CBC_ Hb(Haemoglobin) (g/dl)

MCV (fl)	**CE INTERPRETATION**							
	Beta Thal Trait	**HbD Punjab**	**HbE Beta Thal**	**HbS**	**HbS Trait**	**S Beta Thal**	**Normal**	**Grand Total**
35-45	2	-	-	-	-	-	-	2
45-55	3	-	-	-	-	-	1	4
55-65	26	-	1	-	1	1	12	41
65-75	12	-	-	1	-	-	12	25
75-85	1	2	-	-	-	-	12	15
85-95	1	1	1	-	1	-	6	10
95-105	-	-	-	-	-	-	2	2
105-115	-	1	-	-	-	-	-	1
Grand Total	45	4	2	1	2	1	45	100

TABLE 4 - CE Interpretation with CBC_MCV (Mean corpuscular volume) (fl)

MENTZER INDEX	**CE INTERPRETATION**							
	Beta Thal Trait	**HbD Punjab**	**HbE Beta Thal**	**HbS**	**HbS Trait**	**S Beta Thal**	**Normal**	**Grand Total**
<13	40	1	-	-	1	1	6	49
13 & Above	5	3	2	1	1	-	39	51
Grand Total	45	4	2	1	2	1	45	100

TABLE 5 - CE Interpretation with MENTZER INDEX

Quant_HbA(%)	CE INTERPRETATION							
	Beta Thal Trait	HbD Punjab	HbE Beta Thal	HbS	HbS Trait	S Beta Thal	Normal	Grand Total
0-20	-	-	-	1	-	1	-	2
40-60	-	2	-	-	1	-	-	3
60-80	-	2	1	-	1	-	-	4
80-98	45	-	1	-	-	-	45	91
Grand Total	45	4	2	1	2	1	45	100

TABLE 6 - CE Interpretation with Quant_Hb A (%)

Quant_HbA2 (%)	CE INTERPRETATION							
	Beta Thal Trait	HbD Punjab	HbE Beta Thal	HbS	HbS Trait	S Beta Thal	Normal	Grand Total
1-2	-	1	-	-	-	-	7	8
2-3	-	-	-	1	2	-	35	38
3-4	1	3	-	-	-	-	3	7
4-5	12	-	2	-	-	1	-	15
5-6	28	-	-	-	-	-	-	28
6-7	4	-	-	-	-	-	-	4
Grand Total	45	4	2	1	2	1	45	100

TABLE 7 - CE Interpretation with Quant_Hb A2 (%)

Quant_HbF (%)	CE INTERPRETATION							
	Beta Thal Trait	HbD Punjab	HbE Beta Thal	HbS	HbS Trait	S Beta Thal	Normal	Grand Total

0-10	45	4	2	-	2	1	45	99
30-40	-	-	-	1	-	-	-	1
Grand Total	45	4	2	1	2	1	45	100

TABLE 8 - CE Interpretation with Quant_Hb F (%)

DISCUSSION

Haemoglobinopathies are conventionally classified into two main categories, which entail structural Haemoglobin variants (i.e. qualitative or functional disorders) and thalassemias (i.e. quantitative disorders, characterized by defective globin production). Approximately 7% of worldwide population carries an inherited disorder, and upto 5,00,000 babies are born each year with severe forms of these conditions.[4]

In India, the sickle cell gene is mostly found among the Dravidians & Pre-Dravidian tribes. Lehman and Cutbush (1952)[5] were the first to report the presence of the sickle cell gene in the tribal groups of Nilgiri hills in South India. Haemoglobin E is common in South-east Asia with gene frequency in some countries ranging from 8% to 70%.[6] It is also found in Sri Lanka, India, Bangladesh, Pakistan, Nepal, Vietnam, Malaysia, Philippines, Indonesia and Turkey. HbE is the commonest haemoglobin abnormality seen in north-east India, especially West Bengal and Assam.[6] Beta (β) thalassemia is detectable in almost every Indian population; however, it is seen with highest frequency in north-west and far East. Sindhis, Gujaratis, Bengalis, Punjabis and Muslims account for most of β Thalassemia.[7] Carrier state for (β) thalassemia in India varies from 1-17% with an average of 3.2%.[8]Alpha (α) thalassemia is most widely prevalent in the tribal population with a frequency of 1-40% in Andhra Pradesh and Gujarat.[9]The inherited disorders of haemoglobin synthesis are one of the important public health problems in India. India is the home of several haemoglobin variants. Haemoglobinopathies are inherited disorder of globin chain synthesis. It either reduces rate of synthesis or structurally abnormal globin chain leading to abnormal haemoglobin molecule synthesis. The diagnosis of haemoglobinopathy can result from either clinical suspicion or from follow up of an abnormality detected during screening.[10]

In this study, total 100 cases of reproductive age groups were studied. The most common haemoglobinopathy in this study is β thalassemia trait (45%) followed by HbD Punjab (4%), Sickle cell trait (2%), HbE Beta thal (2%), HbS (1%) and S Beta thal (1%). Colah et al[11]

reported that nearly 1.5% of the world's population is carriers of β thalassemia. The overall gene frequency of β Thalassemia trait reported in northern and western India was 4.05%.[12] In central India, the prevalence of β thalassemia trait has been estimated to be 9.59%.[13] These data reveal that in most parts of India, β thalassemia trait is the commonest disorder.

Out of 100 cases, 45% were normal and 55% cases had abnormal haemoglobin pattern. Of the 55 abnormal cases, 38% were males and 17% were females, thus giving slight male preponderance. This may be due to the prevalent socio-cultural factors in our society, that more male patients seek medical attention. As the study was conducted in reproductive age group, maximum number of cases were in 25-34 years of age (30%) followed by 35-45 years of age (15%) and 15-24 years of age (10%). Majority of the diagnosed individuals belonged to the Hindu religion (36%) while 12% were Muslims, 5% were Punjabis and 2% were Sindhis. Of the 100 cases of anemia on peripheral blood film, maximum 33% shows microcytic hypochromic RBC with anisopoikilocytosis. The most common cause of microcytic hypochromic anemia is iron deficiency anemia. It is obligatory on physicians to first rule out Iron deficiency anemia and then investigate for haemoglobinopathies, otherwise the overenthusiatic approach inflates the cost of treatment for the patient. In this study, abnormal haemoglobin pattern was seen in 20% cases of microcytic hypochromic anemia. Hence, MCV is a key diagnostic indicator. Thalassemic individuals have a reduced MCV, and Lafferty JD et al[14] suggested that an MCV of 72 fl is maximally sensitive and specific for presumptive diagnosis of thalassemia syndromes. In this study, out of 55 diagnosed cases, 29% of the cases have low MCV in the range of 55-65 fl. Mondal et al[15] in 2014 studied the spectrum of thalassemia and haemoglobinopathies in West Bengal. Total 90,210 cases were included and 10,313 cases (11.43%) shown abnormalities. β thalassemia trait was the most common abnormality found in 3870 cases (4.29%). Based on this study also, β thalassemia trait is far more common than rest of thalassemia and haemoglobinopathies in India and a detailed population screening is required to find out the exact prevalence of β thalassemia trait.Rasouli Ghahfarokhi SM et al[16] in 2015 studied 19.5% of the individuals were classified as beta thalassemia carriers. 3.3%, 2.5%, 1.5% and 0.5% of the subjects were heterozygote for Hb S, Hb D, Hb C and Hb barts, respectively. Thalassemia is an autosomal recessive disorder which is inherited from parents. As β thalassemia follows the Mendelian law so a pre marital check up of both males and females should be carried out to avoid the burden of β thalassemia major in the society. The numbers approximately were similar

to the study performed by Joshaghani and colleagues in North of Iran. In that study, Hb electrophoresis was carried out by capillary electrophoresis and 0.27%, 4.68%, 55%,0.27% and 0.41% were recorded for Hb E, Hb D, Hb S, Hb H and Hb Bart, respectively.[17] In our study, the numbers for other haemoglobinopathies were HbD Punjab (4%), Sickle cell trait (2%), HbE Beta thal (2%), HbS (1%) and S Beta thal (1%).

A joint effort by all the State Governments supported by theCentre along with help from NGOs, thalassemia societies andcorporate houses as a part of their social responsibilities andstrongly backed by political would be required for successfulimplementation of a national control programme. Guidelines forthis have recently been prepared by the National HealthMission, Ministry of Health and Family Welfare with the help ofseveral experts in the country(National Health MissionGuidelines on Hemoglobinopathies in India 2016).[18]

Capillary haemoglobin electrophoresis has been shown to be a reliable alternative to High performance liquid chromatography (HPLC) and is one that provides a more user-friendly interpretive format. It offers high resolution, automated sampling and data collection, low sample volumes and low reagent use and cost. It is a revolutional investigation which can easily take to remotest corner of the country & can be used as a screening as well as diagnostic modality for thalassemia and haemoglobinopathies. The term “prevention is better than cure” is apt for these conditions.

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TABLES WITH LEGENDS:

TABLE 1: CE Interpretation with Religion.

TABLE 2: CE Interpretation with Chief Complaints.

TABLE 3: CE Interpretation with CBC_ Hb (Haemoglobin) (g/dl).

TABLE 4: CE Interpretation with CBC_MCV (Mean corpuscular volume) (fl).

TABLE 5: CE Interpretation with Mentzer Index.

TABLE 6: CE Interpretation with Quant_Hb A (%).

TABLE 7: CE Interpretation with Quant_Hb A2 (%).

TABLE 8: CE Interpretation with Quant_Hb F (%).

GRAPHS WITH LEGENDS:

GRAPH 1: Age Distribution

GRAPH 2: Gender Distribution

GRAPH 3: Distribution according to Family history.

GRAPH 4: Distribution according to Marital Status.

GRAPH 5: Distribution of cases according to PBF.

GRAPH 6: CE Interpretation