Research Article

Prevalence and spectrum of hemoglobinopathies in tertiary care centre in a rural area of Madhya Pradesh

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ABSTRACT

Background: Haemoglobinopathies like thalassaemia and sickle cell anaemia etc are increasing due to unawareness of rural population. This study indicates type of haemoglobinopathies amongst the patients of a rural based tertiary care hospital in one year and nine months.
Methods: Five hundred ten patients were studied during last one year and nine month for all suspected cases of haemolytic anaemia based on Complete Blood Count, Red cell indices and Peripheral blood smear examination. Sickling test, test for Hb F and haemoglobin electrophoresis with quantification of bands are done in all these cases
Results: Out of all 510 cases of anaemia 461 cases (90.39%) were confirmed to nonhaemolytic anaemia whereas 49 cases (9.60%) had shown abnormal haemoglobin bands on electrophoresis. Out of these 49 cases 29 (59.18%) were Males and 20 (40.81%) were females. Most common Haemoglobinopathy observed was Sickle cell β Thalassaemia 23 (4.50%) followed by β Thalassaemia Trait 9 (1.76%), Sickle Cell trait 7 (1.37%). β Thalassaemia Major 5 (0.98%) & Sickle Cell Disease 5 (0.98%) have equal prevalence. The onset of disease was most prominent in Neonatal to pediatric age group including early adolescent (0-18 years) followed by reproductive age group (19- 45 years). Few cases of old age (46+ years) were detected.
Conclusion: Study provides data on the spectrum & pattern of Haemoglobinopathies in a rural tertiary care centre. Screening of all anemic patients should be done for Haemoglobinopathy and proper Genetic counseling must be given to all cases to prevent incidence of cases in future generation.

Keywords: Haemoglobinopathy, Haemoglobin electrophoresis, Sickle cell anaemia, Thalassaemia

INTRODUCTION

Haemoglobinopathies like thalassaemia, sickle cell anaemia etc are increasing trend due to unawareness of rural population. The General incidence of thalassaemia trait & sickle cell Haemoglobinopathy in India varies between 3-17% & 1-44% respectively.[1]

Though the tribal communities constitute a major part in India, unfortunately they are highly vulnerable to many hereditary disorders causing high degree of morbidity & mortality. Haemolytic anaemia are one of such disease and due to unknown reason some geographical area & races show very high incidence making the haemoglobinopathies, a major public health problem in our country.[1,2] Haemoglobinopathies are also an important causes of morbidity and Mortality worldwide. Originally Sickle cell disease was confined to Africa, Mediterranean basin, the middle east and India, whereas thalassaemia gene are common in west and central Africa, the mediterranean basin, the middle east, Asia, China & the pacific island. Nevertheless nowadays they are widespread throughout the world because of increasing migration.[3] These cases can be treated by bone marrow transplantation.

Greece, Cyprus & Italy have attained a success in preventing these diseases by population screening, Genetic counseling and prenatal diagnosis. All case of anemia subjected to be investigated by CBC & peripheral smear, and then all these cases are subjected to special investigations. Affected person and their families with haemoglobinopathies must be offered genetic counseling. This study is to analyze the types of haemoglobinopathies amongst patients of Anaemia in last one year and nine month in a rural belt in Madhya Pradesh.

METHODS

A total 510 patients of clinical anaemia during last one year and nine month (Nov 2011-Aug 2013) were subjected to various investigations. Two ml of Intravenous Blood sample was collected from all cases after obtaining informed consent using Ethelene Diamine Tetraacetic Acid (EDTA) as anticoagulant in vacutainers. Patients who received blood transfusion within 4 weeks were deferred till 4 weeks after transfusion. Haemolysate with a concentration of 1.6g/dl (in ratio of 1:5) was prepared for haemoglobin (Hb) electrophoresis.[4] Complete blood count (CBC) Blood cell indices were measured using Sysmex XS- 800i fully automated blood cell counter & Cellenium 19 which was calibrated with commercially available control.

As a special investigation the Alkali denaturation test for fetal haemoglobin & sickling test was performed using freshly prepared sodium metabisulphite solution as a reducing agent.[5] Hemoglobin electrophoresis was performed using Inter Lab Genio S electrophoresis apparatus and commercially available Interlab Master Kit at PH 8.6 (Figure 1).

Figure 1: Showing Interlab Genio S electrophoretic apparatus.

This kit provides the electrophoresis separation of haemoglobin on a cellulose acetate strip. After electrophoresis, different haemoglobin types forms different band on cellulose acetate strip. These bands are then read by densitometer automatically by the electrophoretic apparatus (Figure 2 & 3).

Figure 2: Showing Bands of different haemoglobin on cellulose acetate strip on alkaline gel electrophoresis (Band of HbA, HbS, HbF, and HbA2).
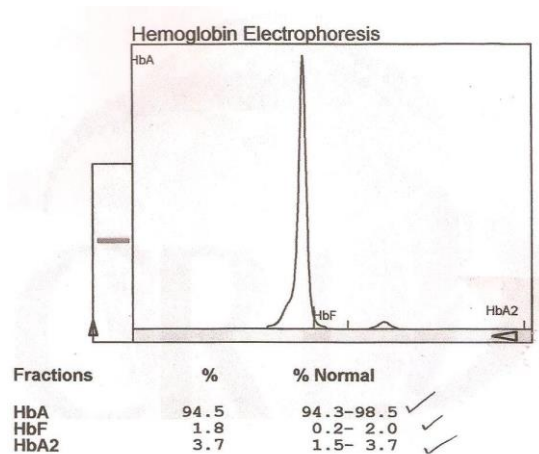
Figure 3a

Fractions	%	% Normal	
HbA	94.5	94.3-98.5	✓
HbF	1.8	0.2- 2.0	✓
HbA2	3.7	1.5- 3.7	✓

Figure 3b

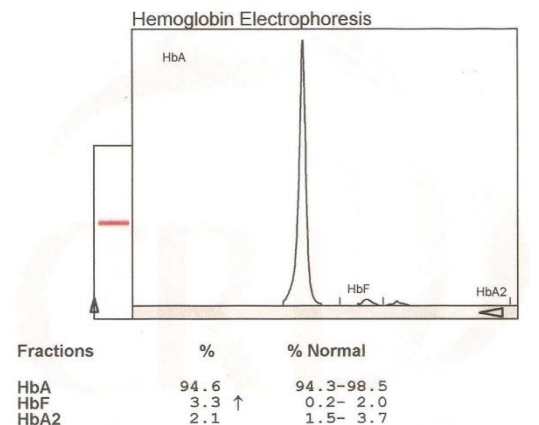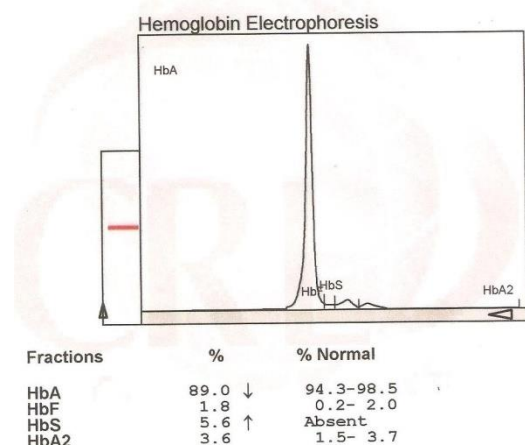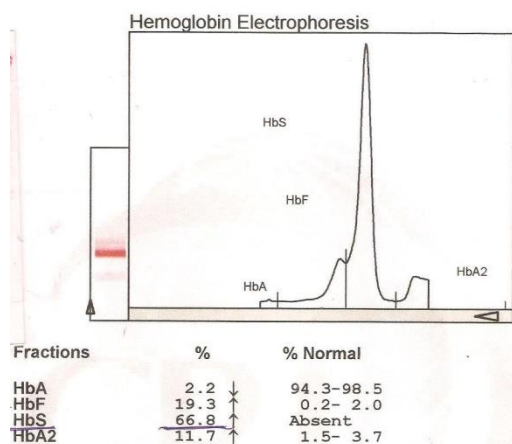| Fractions	%	% Normal
HbA	94.6	94.3-98.5
HbF	3.3 ↑	0.2- 2.0
HbA2	2.1	1.5- 3.7

Figure 3c

Figure 3d

Figure 3: Showing densitometry scan of different fraction of Hb (Figure 3a) showing scanning of normal Hb bands, (figure 3b) showing scanning of different bands of Hb of β Thalassaemia trait, (figure 3c) showing scanning of different bands of Hb of Sickle cell trait & (figure 3d) showing scanning of different bands of Hb of Sickle cell β Thalassaemia).

A value more than 3.5% of HbA2 fraction of Hb was taken as cut off point for determining the β thalassaemia trait & more than 10% was assumed to be HbE.[6]

RESULTS

461 cases (90.39%) out of all 510 cases detected to have nonhaemolytic anaemia whereas 49 (9.60%) cases had shown abnormal Haemoglobin. All 510 cases of clinical anaemias were included in the study. They were haematologically evaluated for evidence of haemolytic anaemia at Department of Pathology. Out of all the 510 cases 461 cases (90.39%) were confirmed to have nonhaemolytic anaemia whereas 49 (9.60%) cases had shown abnormal haemoglobin bands on electrophoresis

indicating haemolytic anaemia. These 49 cases consisted of 29 males (59.18%) & 20 females (40.81%). Thus having male prepondence.

Further analysis as shown in Table 1, reveals prevalence of disease is 69.38% in paediatric age group Table 1 showing distribution of haemoglobinopathies in adult and paediatric age group.

Table 1: Adult and pediatric age group (Including early adolescents) distribution of different haemoglobinopathies.

Age groups	Male (n=29)	Female (n=20)	Total (n=49)
0- 18	23 (79.31%)	11 (55%)	34 (69.38%)
19- 45	03 (10.34%)	09 (45%)	12 (24.48%)
46 yrs & above	03 (10.34%)	00 (00%)	03 (6.12%)

Clinical presentations of 510 anaemic patients are illustrated in Table (2). Pallor was the most prevalent manifestation & was present in 492 cases (96.4%) out of total 510 anaemic patients whereas the haemolytic facies were seen in 40 (7.8%) cases.

Table 2: Pattern of clinical presentation in anaemic patients (n=510).

Features	Numbers (%)
Pallor	492 (96.4)
Icterus	75 (14.7)
Splenomegaly	130 (25.4)
Hepatomegaly	99 (19..4)
Ascites	15 (2.9)
Haemolytic Facies	40 (7.8)
Bone Pain	102 (20)
Oedema	95 (18.6)

The spectrum of haemoglobinopathies prevalent in patients of rural population is shown in Table 3 in one year and nine month. Sickle-thalassaemia is the most common haemoglobinopathy 23 (4.50%), followed by β Thalassaemia trait 9 (1.76%). Other haemoglobinopathies in decreasing order were Sickle Cell Trait 7 (1.37%), β Thalassaemia Major & Sickle Cell Disease has equal incidence that is 5 (0.98%).

The onset of disease was most prominent in Neonatal to pediatric age group including early adolescent (0-18 years) followed by reproductive age group (19- 45Years). Few cases of old age (46+ years) were detected only

when they faced clinical complications. This study found many variations in the clinical presentation of disease. Patients belonging to β thalassaemia minor group had mild anaemia with decreased total Hb and HbA compare to normal. Haemoglobin electrophoresis showed a slight increase in HbA2 and HbF. It is postulated that these patients inherit one gene of β -thalassaemia, they are either asymptomatic or develop mild to moderate anaemia. In this study the red blood cells showed hypochromic and microcytic picture. Genetic counseling for couple at risk, for offspring with homozygous β -thalassaemia should be done in these patients.[7]

Table 3: Spectrum of haemoglobinopathy.

	Male	Female	Total	Percentage
Non-haemolytic anaemia	257	204	461	90.39
Sickle cell trait	5	2	7	1.37
Sickle cell disease	3	2	5	0.98
Sickle cell - β thalassaemia	13	10	23	4.50
β-Thalassaemia trait	5	4	9	1.76
Thalassaemia major	3	2	5	0.98
Total	286	224	510	100

DISCUSSION

Haemoglobinopathies are inherited disorder of globin chain synthesis. It either reduced rate of synthesis or structurally abnormal globin chain leading to abnormal haemoglobin molecule synthesis. The diagnosis of haemoglobinopathy including thalassaemia can result from either clinical suspicion or from follow up of an abnormality detected during screening.[8] In our study screening of all anaemia cases was done initially by clinical history including family history, cast & ethnicity of the patients. All cases were subjected to physical examination, a blood count & peripheral blood examination. In addition Sickle cell phenomenon & Alkali denaturation for foetal haemoglobin was also carried out. The more confirmatory test by Cellulose Acetate Membrane electrophoresis at alkaline pH (CAM) was followed in all cases. Various Indian studies have reported that many variants of haemoglobin are prevalent and very common in rural Indian population. Haemoglobinopathies are one of the major public health problems in our country. On the basis of analysis of reports published in last 20 years it is observed that several tribes in various parts of India have been identified as high risk groups of Haemoglobinopathies. In India about 4635 ethnic communities have shown 05 common & 12 rare mutations.[9]

The prevalence of thalassaemia has been reported by various authors from 0-10.5% in different caste/ ethnic groups. In our study the thalassaemia constituted (2.74%)

of all haemoglobinopathies. In one of the recent study on thalassaemia in tribal population of India, the Madhya Pradesh including Malwa region amongst the Bhil, Gond & Pawar tribes have high incidence. In our study we found that Bilala tribe in the area of study, has very high incidence of haemoglobinopathies. The frequency of thalassaemia is increased by consanguity & endogenous mating , it may be assumed that tribal community in India are facing the problem at large scale.[9] Chopra et al[10] has reported, β thalassaemia trait 17% & β thalassaemia major 0.4% amongst armed forces personnel & their families which constitutes a mixed India ethnic population. In another recent study the prevalence of haemoglobinopathy has been reported as high as 42.2%, with most common haemoglobinopathy observed was β thalassaemia trait 21.3%. The authors have observed a very high incidence of haemoglobinopathies in paediatric age group (0-18 years) as 55.7%.[7] This is very well correlated with our study which has revealed maximum prevalence of 69.38%.

There are so many disorders of Red blood cells but haemoglobinopathy are most common disorder. Most common haemoglobinopathy in this study was sickle cell β thalassaemia [4.50%] followed by β thalassaemia trait [1.76%] & Sickle cell trait [1.37%]. Thalassaemia major [0.98%] & Sickle cell disease [0.98%] has equal & lowest prevalence. The prevalence of β thalassaemia major & sickle cell disease was low [0.98%] & all their families given genetic counseling.

This study provides a data on spectrum of haemoglobinopathy in rural based tertiary care hospital (Index Medical Collage & Hospital) for the first time.

All High risk patients with anemia should be screened for HbA$_2$. We can prevent the haemoglobinopathies in this area by genetic counseling & premarital HbA2 estimation.

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