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ORIGINAL ARTICLE

Hemoglobinopathies in Dharwad, North Karnataka: A Hospital-Based Study

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ABSTRACT

The inherited disorders of hemoglobin are responsible for an extremely complex series of clinical phenotypes. Sickle cell anaemia and thalassemia can cause chronic ill-health and life-threatening situations. Present study was carried out at Dharwad of North Karnataka. The practice of consanguineous marriages is an accepted socio-cultural phenomenon in this region. This study was hospital-based and the paediatric cases of hemoglobinopathies were identified based on clinical data, family history, red blood cell indices and hemoglobin electrophoresis. Out of the fifty cases , twenty chidren were carriers of beta- thalassemia trait and fifteen children were suffering from beta-thalassemia major. Two cases of sickle cell trait and lone case of a compound heterozygote for HbS/beta-thalassemia were also identified. Families of four cases of hemoglobinopathies were studied in detail to identify the carriers of abnormal hemoglobins. Ten out of fifty children of the study were products of consanguineous mating. The population of Dharwad appears to be a repository of thalassemia. An extensive screening of the population is needed to assess the prevalence of hemoglobinopathies, which will help in identification of carriers of hemoglobinopathies and further in taking adequate therapeutic and preventive measures.

Key messages :

1. Hemoglobinopathies pose economical and psychosocial burden
2. Extensive screening for hemoglobinopathies is very much essential
3. Socio-cultural aspects of a region need to be explored to evaluate their interaction with genetic factors.

Key Words : Abnormal Hemoglobins, Compound Heterozygote, Family Studies, Hemoglobinopathies, Hemoglobin Electrophoresis, Pedigree, Red Cell Indices, Sickle-cell trait, Thalassemias

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Introduction

The inherited disorders of hemoglobin are the commonest single-gene disorders in man. They fall into three overlapping groups : structural variants; thalassemias characterized by reduced rate of synthesis of one or more globin chains; and conditions in which fetal hemoglobin synthesis persists beyond the neonatal period, collectively known as hereditary persistence of fetal hemoglobin. Hemoglobin disorders are responsible for an extremely complex series of clinical phenotypes [1].

The World Health Organization (WHO) has suggested that about 5% of the world population are carriers for different inherited disorders of hemoglobin [2]. WHO reports also state that about 370,000 severely affected homozygotes or compound heterozygotes of thalassemia are born every year. The UNICEF in 1996 estimated that there were 29.7 million carriers of beta thalassemia trait in India and about 10,000 infants with homozygous beta thalassemia born every year [3]. The general incidence of thalassemia trait and sickle cell hemoglobinopathies in India

varies between 3-17% and 1-44% respectively [4], [5],[6]. It is estimated that there are about 65,000-67,000 beta-thalassemia patients in India with around 9,000-10,000 cases being added every year. The carrier rate for beta-thalassemia gene varies from 1 to 3% in Southern India to 3 to 15% in Northern India[7],[8],[9].

In developing countries , in which there is high mortality from infections and malnutrition in the first year of life, many of the hemoglobinopathies are unrecognized.

Sickle cell anaemia and thalassemia major can cause life-threatening situation and chronic ill health. They pose economical and psychological burden on the affected individual and his/her family, and the society as a whole. Hence, the population needs to be screened for hemoglobin.

Materials and Methods

Source of Data :

1. Children with anaemia, generalized weakness, fever and splenomegaly, visiting the Paediatrics O.P.D. of the hospital ;
2. Childern with severe hemolytic anaemia, hepatosplenomegaly and history of blood transfusion, and admitted to the Paediatrics ward of the hospital.

Above children were aged between 3 months to 15 years (n = 50), and were referred to the departments of Biochemistry and Pathology for laboratory investigations of hemoglobinopathies.
Our research protocol was approved by the Ethical Committee of the institution. Voluntary consent was obtained from the subjects of the study. Detailed family history was collected from the patients / guardians regarding previous history of hemolytic anaemia and blood transfusions, any medications taken, consanguinity in marriages, and clinical signs and symptoms.Reproductive history of the parents with regard to abortions, death of any child, still birth was collected.

Laboratory investigations : 5 ml. of blood was collected in vacutainer tubes having EDTA as anticoagulant.

a) Hematological parameters : Hb, PCV, MCV, MCH, MCHC, RBC count and WBC count were measured using Sysmex cell counter [10]. Peripheral smear was evaluated for features of red cell morphology [10] .

disorders so that appropriate measures for treatment and prevention can be taken.

The present study was a pilot study carried out in S.D.M.College of Medical Sciences and Hospital, Dharwad, situated in Northern Karnataka. To the best of our knowledge, it is the first of its kind reported from North Karnataka. This region has unique socio-cultural practices.The practice of consanguineous marriages preferably with maternal kindreds, is an accepted socio-cultural phenomenon irrespective of religion, caste and economic status. The study aimed to identify the children with hemoglobin disorders, using the available hematological and biochemical tests. We also attempted to make a detailed family study of thalassemia patients, to evaluate the inheritance patterns and to identify thalassemia carriers.

b) NESTROFT (Naked Eye single tube red cell osmotic fragility test) was performed to screen the cases of beta-thalassemia trait [9,10].
c) Hb electrophoresis : The red cell hemolysate was diluted to have Hb concentration of 10µg/10µl. Electrophoresis was carried out at alkaline pH of 8.6, on cellulose acetate strips, at 450V for 40 minutes. After the run, the strips were stained in Ponceau S. Interpretation of the migration pattern of the test samples was undertaken by comparing them to those of known controls obtained from Helena Bioscienecs, London. Location of HbF was confirmed by performing electrophoresis with cord blood [10], [11].
d) Estimation of HbA_2 : After the run of electrophoresis, the HbA_2 and HbA bands were eluted from the cellulose strip and their absorbances were read at 415 nm, using which HbA_2 % was calculated [10].
e) Estimation of HbF : HbF% was measured by alkali denaturation method of Betke [10,11].
f) Test for unstable hemoglobins : Stability of Hb in isopropanol,was tested [11] .
g) Test for HbS : Solubility of deoxygenated Hb in saponin-phosphate buffer was tested and then HbS% was calculated [10],[11].

FAMILY STUDIES : Family studies of four patients with hemoglobinopathies, were done. Out of them, two were cases of beta thalassemia major,

one of beta thalassemia trait, and one case of a compound heterozygote of HbS/beta thalassemia . The blood samples of available members of the family tree were collected and subjected to hematological and biochemical investigations of hemoglobinopathies.

ANALYSIS OF DATA: Diagnosis of hemoglobinopathies was made based on the hematologic parameters, laboratory tests and clinical findings, as mentioned in standard literature [4,10,11] . The values of hematologic parameters were expressed as mean. Pedigree diagrams were drawn based on the family studies [12].

Results

The results of the present study are presented in the Table/Fig 1 to Table/Fig 6. Fifty children aged between 3 months and 15 years, were the subjects of our study. They were suspected cases of hemoglobinopathies, and referred for laboratory investigations of hemoglobinopathies. There were 15 children with beta thalassemia major, 20 with beta thalassemia trait, two with sickle cell trait and one case of a compound heterozygote of HbS/beta thalassemia trait. Out of the fifty children, thirty five were males and fifteen were females. Out of the total fifteen cases of thalassemia major, twelve cases (80%) were males and only three (20%) were females. In case of twenty children with thalassemia trait, thirteen (65%) were males and seven (35%) were females. Seven children showed normal patterns of hemoglobin electrophoresis and none of the laboratory parameters indicated any hemoglobinopathy in them. In five cases, the laboratory and clinical data were insufficient to confirm the diagnosis. Beta-thalasemia trait was characterized by mean Hb of 8.1 g%, mean MCV of 71fl, mean MCH of 21pg, HbA_2 value of 8%, and positive NESTROFT. In beta-thalassemia major cases, Hb was 5.5g%, MCV was 62fl, MCH was 18pg, HbA_2 was 3%, and HbF was 55%. Beta-thalassemia major cases were also characterized by severe microcytic hypochromic anaemia, anisocytosis, poikilocytosis and high percentage of target cells, as demonstrated by peripheral smear study. Sickle cell trait cases showed positive solubility test and HbS value of 25%.

Table /Fig 1
Hematological parameters and laboratory findings in children aged 3 months to 15 years

Groups	Age & sex	Hb gm%	MCV fl	MCH pg	HbA_2 %	HbF %	HbS %	Electrophoresis Pattern #
Normal (n=7)	3 mths- 15 yrs 2 male; 3 female	12 * (11-14)≠	90* (85-93)≠	31* (29-34)≠	2.5* (2-3)≠	0.8* (0.5-1.0)≠	-----	A_2, A
β-thalassemia trait (n=20)	3 mths-15 yrs 13 male;7 female	8.1* (7-10.5)≠	71* (65-80)≠	21* (19-25)≠	8* (4-10)≠	8* (4-15)≠	-----	A_2, F, A
β-thalassemia major (n=15)	3 mths-15 yrs 12 male; 3 female	5.5* (3.5-6.2)≠	62* (52-67)≠	18* (14-20)≠	3* (2-5)≠	55* (38-75)≠		A_2, F, A
Sickle cell trait (n=2)	a)14 yrs male b) 9 yrs male	9.2 9.4	85 83	27 28	2 3	1 1	26 24	A_2, S, A A_2, S, A
HbS/ β-thalassemia trait (n=1)	6 yrs male	8.5	72	22	5	8	60	A_2,S, F, A

* Values presented as mean ≠ Range of the values given # Bands on cellulose acetate strip, from anodal to cathodal direction

Table/Fig 2
Hematological parameters and laboratory findings in five un-confirmed cases; children aged 3 months to 15 years

Sl.no.	Age & sex	Hb gm%	MCV fl	MCH pg	HbA_2 %	HbF %	HbS %	Electrophoresis Pattern#
1	5 yrs male	11.5	74	23	1.0	1.0	-----	A_2, A
2	5 yrs male	11	75	23	2.0	1.5	-----	A_2, A
3	8 yrs male	10	78	26	2.0	5		A_2, F, A
4	13 yrs male	10	79	26	1.5	4	----	A_2, F, A
5	6 yrs male	12	78	27	3	5	----	A_2, F, A

Bands on cellulose acetate strip, from anodal to cathodal direction.

In compound heterozygote of HbS/beta-thalasemia, HbS was 60% and this case was confirmed by electrophoresis, HbF and HbA_2 estimations. Characteristic electrophoretic patterns were observed in all the confirmed cases of hemoglobinopathies.
As per the family history collected, 10 out of the 50 children of our study were the products of consanguineous marriages. Out of these, six were beta thalassemia major-children, two were beta thalassemia carriers and one was a compound heterozygote of HbS/beta thalassmia, and one was an unconfirmed case of hemoglobinopathy. Six thalassemic children had history of blood transfusions.

Discussion

Hemoglobinopathies are of world-wide occurrence, though some geographical areas have high prevalence of these disorders. In India, average frequency of sickle cell gene is around 5%. The highest frequency of sickle cell gene in India is reported in Orissa (9%), followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) and Gujarat (6.4%) [8-10]. The distribution of beta thalassemia is not uniform in Indian subcontinent. The highest frequency of beta thalassemia trait is reported in Gujarat (10-15%), followed by Sindh (10%), Punjab (6.5%), Tamil Nadu (8.4%) and Maharashtra [4], [5], [6], [13], [14], [15], [16], [17].

In India, the problem of hemoglobinopathies is compounded by the heterogeneity of population. The different regions of India have different gene

frequencies for the various hemoglobinopathies. The fertility rates, the literacy rates, and the rates of consanguineous marriages are also diverse. Northern part of Karnataka comprising the districts of Dharwad, Gulbarga, Bidar, Belgam, Bijapur and Bellary, has unique socio-cultural practices. Consanguinity in marriages is a well-accepted social norm irrespective of religion, caste, educational status and economical back ground. Our's was a pilot study carried out in Dharwad and it was hospital-based. For the family studies of four cases, we visited the houses of the patients.

Diagnosis of hemoglobinopathies was made using the criteria mentioned in standard literature. 30% of the children (subjects of the study) were victims of beta thalassemia, 40% were carriers of beta thalassemia, 4% (2) were cases of sickle cell trait, and one was a compound heterozygote of HbS/beta thalassemia. In five cases, the diagnosis could not be confirmed as the available clinical and laboratory data were insufficient [Table/Fig 2]. These could be the cases of alpha-thalassemia or fusion chains such as delta-beta thalassemia, this conclusion being based on hematologic findings given in previous literature [1],[4] however, diagnosis needs to be confirmed with genetic tests and globin chain analysis.

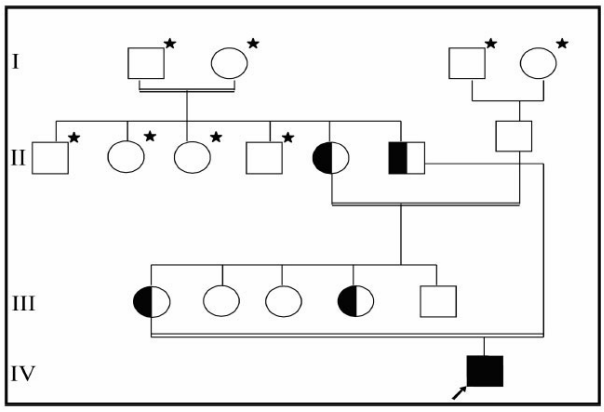
Few reports from India are available regarding the prevalence of compound heterozygotes of thalassemia [18],[19],[20] . According to Garewal

and Das [21], most patients with beta thalassemia are compound heterozygotes having inherited two different mutations, one from each parent. Consanguinity is known to play an important role in hereditary diseases, particularly in autosomal recessive traits. 20% (10 out of 50) of the cases in our study were offsprings of consanguineous marriages. Five children were offsprings of third degree consanguineous mating (marriage between uncle and niece, which is very common in North Karnataka), and five children were offsprings of fourth degree consanguineous mating (marriage between first cousins) [22]. Since closely related individuals have a higher chance of carrying the same alleles than less closely related individuals, the children from consanguineous marriages are more frequently homozygous for various alleles than are children from non-consanguineous marriages[22]. Due to the strict practice of caste endogamy and consanguinity in marriages, association of thalassemia with structural variants of Hb such as E,C and S, is highly prevalent in India [20].

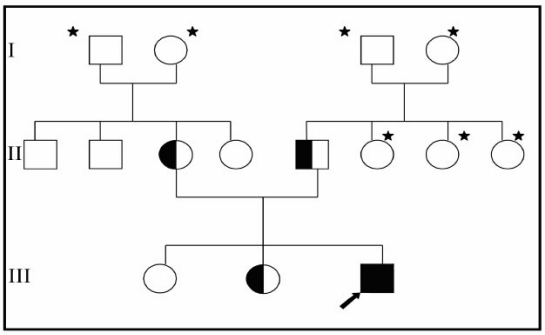
We made an attempt to make a detailed study of thalassemia carriers, inheritance patterns of hemoglobinopathies and further with an aim to help in genetic counseling. We could visit only four families of thalassemia patients due to non-availability of family members during the course of the study, migration of some of the families, hesitation of the socio-economically backward families to participate in the study, and certain misconceptions of illiterate people regarding blood disorders and marriages.

Conclusions

The population of Hubli-Dharwad appears to be a repository of thalassemia. We plan to undertake extensive screening of the population for hemoglobin disorders, with further aim of carrying out regular new born screening and prenatal diagnosis of hemoglobinopathies, which would go a long way in helping in genetic counseling. The unique socio-cultural practices of North Karnataka need to be explored for their interaction with genetic factors and their role in human health.



Table/Figure 3
Pedigree diagram of a family of a three year-old male child with beta thalassemia major. Proband is the product of consanguineous mating



Table/Fig 4
Pedigree diagram of family of two-year male child beta-thalassemia major

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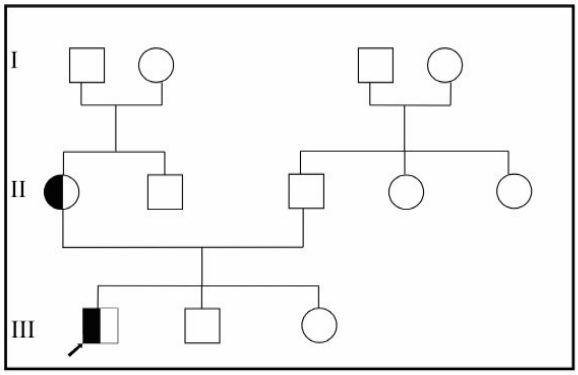
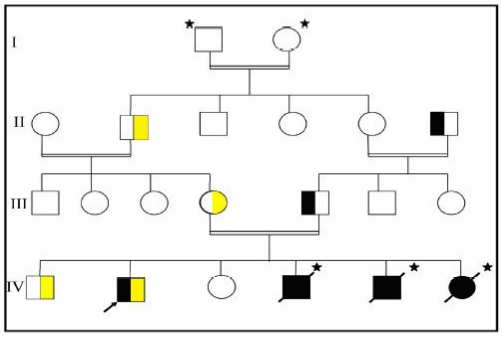
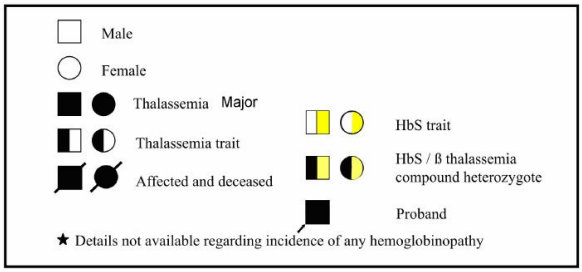


Table/Fig 5
Pedigree diagram of family of a five year - old male child with beta-thalasemia trait



Table/Fig 6
Pedigree diagram of family of a two year-old male child with HbS/beta-thalassemia. Proband is the product of consanguineous mating.

Symbols used in pedigree diagrams



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