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BIOCHEMICAL CHARACTERISATION OF SPECTRUM OF HEMOGLOBINOPATHIES AND THALASSEMIA SYNDROMES - EXPERIENCE WITH 689 CASES IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

The inherited diseases of hemoglobin have remarkable phenotypic variability because of genetic modifiers necessitating medical intervention at various stages of disease. Genotype–phenotype relationship is crucial in this regard. So three year retrospective study of biochemical pattern of Hemoglobinopathies and Thalassemias and their clinical manifestations was done in a cohort of 689 patients in a tertiary care hospital. The highest incidence was Sickle cell disease (15.8%) followed by Sickle cell trait (13.1%), Thalassemia minor (11.8%), HbS/β-thalassemia (5.7%), Thalassemia major (1.6%). Less frequent were HbH disease, HPFH, Thalassemia intermedia, HbE, HbS/HbD, HbE/β-thalassemia, HbS/HPFH, HbD/β-thalassemia, HPFH/β-thalassemia, δβ trait and HbQ. Males had significantly higher incidence (55.2%). Thalassemia major had reduced HbA2 (1.9%). HbAS/α-thalassemia had higher mean age and low HbS as compared to Sickle cell trait. HbS/β^0-thalassemia had lower mean age and higher HbA2, HbS than HbS/β^+-thalassemia, corresponding with severity of disease. To conclude biochemical characterisation closely correlates with the clinical phenotype enabling correct diagnosis and avoids unnecessary investigations.

KEYWORDS: **Hemoglobinopathies, Thalassemia, Biochemical characterisation, HPLC.**

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INTRODUCTION

Hemoglobinopathies result from a structural defect in the globin gene, and thalassemias from a quantitative defect in the globin chain production. There is an overlap between these groups. Sickle cell disease (SCD) is an inherited chronic hemolytic anaemia. Thalassemias are divided according to which globin chain is produced in reduced amounts. When no globin chain is synthesized at all they are called α^0 or β^0 thalassemias, when it is produced at a reduced rate, designated as α^+ or β^+ thalassemias. Because thalassemia occurs in populations in which structural hemoglobin variants are common, they may receive a thalassemic gene from one parent and a gene for a structural hemoglobin variant from the other. Thalassemia and hemoglobin disorders are one of the common hereditary disorders in our country and cause major morbidity and mortality in India[1] and abroad[2]. The World Health Organization estimates that about 7% of the world populations are carriers[3] and that every year 60,000 thalassemia babies are born all over the world[4]. The frequency of total Hemoglobinopathies in India was reported to be 4.2% with 30 million carriers and 15,000 infants with major hemoglobinopathies[5]. These disorders show remarkable phenotypic variability, ranging from severe life-threatening anemia to an extremely mild condition that may be identified only by chance, because of various genetic modifiers effecting gene expression, reducing the degree of imbalance of globin chain synthesis, that affect the complications of disease. Environmental factors may also play an important role in modifying the disease expression. Hence, biochemical characterisation of the abnormal hemoglobins at the time of seeking medical help in tertiary care hospital is important in understanding the pathophysiology of the disease and enabling prompt and appropriate treatment and avoids unnecessary further investigations. Genotype–phenotype relationship is crucial in this regard. So three year retrospective study of biochemical pattern of Hemoglobinopathies and Thalassemias and their clinical manifestations was done in a cohort of 689 patients in a tertiary care hospital of south India.

MATERIALS AND METHODS

This is a retrospective study in a cohort of patients attending the out-patient department of a hospital in south India. Three years data from June 2010 to June 2013 is taken from the records noting the age, sex, consanguinity, phenotype, concentrations of HbA, HbA2, HbF, HbS and any unknown peak, where possible Hb conc., MCV, MCH and MCHC. Hematological parameters were done with cell counter; quantification of hemoglobin fractions by high-performance liquid chromatography method (BIORAD D10). Family history was taken.

EXCLUSION CRITERIA: Patients below 1 year of age, those who had recent blood transfusions.

INCLUSION CRITERIA: Patients with positive family history, abnormal hemograms, patients coming for premarital checkup and Hb variant analysis.

STATISTICAL ANALYSIS

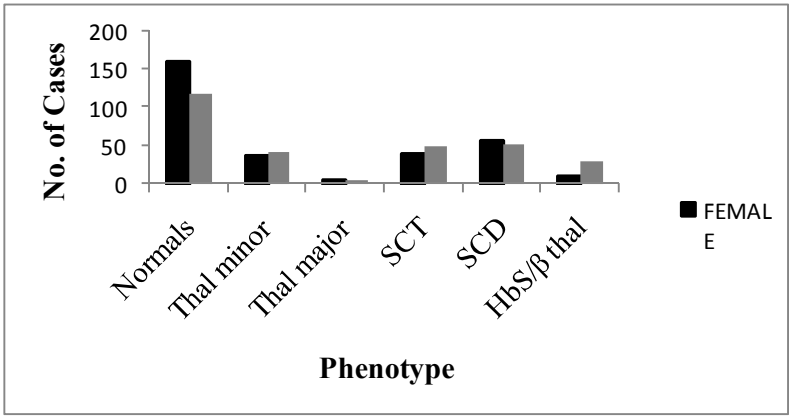
Statistical package for social sciences (SPSS) v. 17.0 was used for Student t-test to calculate statistical significance for the mean age of male and female. ANOVA with multiple comparisons was performed to test the significance of the variables. p value of ≤0.05 was taken as significant.

RESULTS

715 cases, (age range 1-80 years, 95% CI 22-24) were analysed in three years, 689 cases with complete data, were included in the study. Overall, females were 51.5% (N=355) & males were 48.5% (N=334). Out of 689 cases, 40.5% (N=279) were normal & 8.4% (N=58) were cases of Iron deficiency anemia of other causes. 51.1% had abnormal findings on HPLC, major were SCD, SCT, Thalassemia minor, HbS/β thalassemia and Thalassemia major. Males had significantly higher incidence of hemoglobin disorders i.e. 55.2% as compared to 43.2% in females, mainly due to

higher incidence of HbS/β thalassemia (9.0%,N=30) as compared to females 2.5% (N=9), p=0.05. There was no significant difference in the gender preponderance of other phenotypes (Fig 1).

Figure 1
Gender wise distribution of phenotype



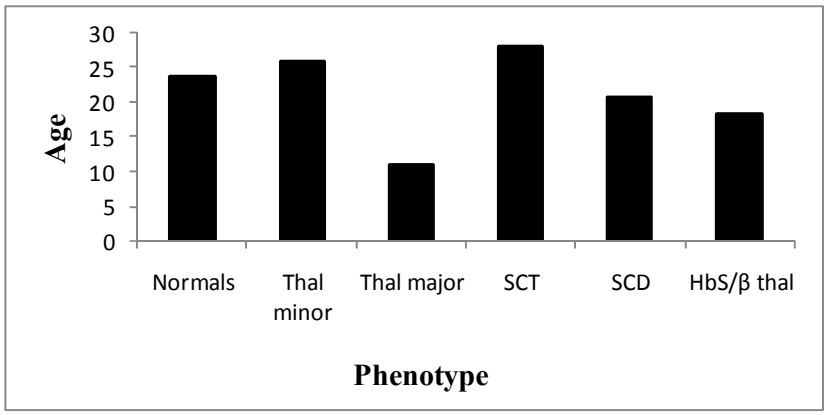
Highest incidence was SCD 15.7% (N=108) followed by SCT 13.1% (N=90), Thalassemia minor 11.8% (N=81), HbS/β-thalassemia 5.7% (N=39) & Thalassemia major 1.6% (N=11). Less frequent were HbH disease 0.6% (N=4), HPFH heterozygous 0.4% (N=3), 0.3% (N=2) each of Thalassemia intermedia, HbE homozygous & HbS/HbD, 0.1% (N=1) each of HbE heterozygous, HbE/β-thalassemia, HbS/HPFH, HbD/β-thalassemia, HbH trait or Hb Barts, HPFH/β-thalassemia, δβ trait, and HbQ. One case, HPFH heterozygous or δβ thalassemia could not be diagnosed for lack of RBC indices (Table1).

Table 1
Incidence of Hemoglobinopathies and Thalassemias

Phenotype	Frequency	Percentage
Normal	279	40.49
Sickle cell disease	108	15.67
Sickle cell trait	90	13.06
Thalassemia minor	81	11.76
IDA	58	8.42
HbS/β thalassemia	39	5.66
Thalassemia major	11	1.6
HbH	4	0.58
HPFH heterozygous	3	0.44
Thalassemia intermedia	2	0.29
HbE homozygous	2	0.29
HbS/HbD	2	0.29
HbE heterozygous	1	0.15
HbE hetero or HbE/β thal	1	0.15
HbS/HPFH	1	0.15
HbD/β thalassemia	1	0.15
HbH hetero/Hb Barts	1	0.15
HPFH/β thalassemia	1	0.15
δβ trait	1	0.15
δβ thal or HPFH hetero	2	0..29
HbQ	1	0.15
Total	**689**	**100**

The mean (±SD) age was 23 yrs. (±12.8). There was no significant age difference between the genders in the major phenotypes. The mean (±SD) age of thalassemia major 12.4(±10.5) and HbS/β-thalassemia (mean±SD=18.8±11.0) was significantly lower than the other phenotypes (mean±SD=25±12.5), p=0.001. Mean age for SCT (mean ± SD=28 ± 14.5) was significantly higher than SCD and HbS/β-thalassemia, p=0.01(Fig 2).

Figure 2
Age of common phenotypes



The levels of HbA and HbF in Thalassemia major were significantly different between males & females (HbA/HbF 19.2%/79% in males against 33.7%/64.2% in females respectively). Mean (±SD) levels of HbA2 were significantly reduced in Thalassemia major (1.9±1.0, $p<0.05$). Mean levels of HbF mildly increased in IDA (2.0±1.7), there was no significant difference between mean values of HbF and HbS between SCD and HbS/β-thalassemia (17.9, 68.6 and 15.6, 63.8) respectively (Table 2)

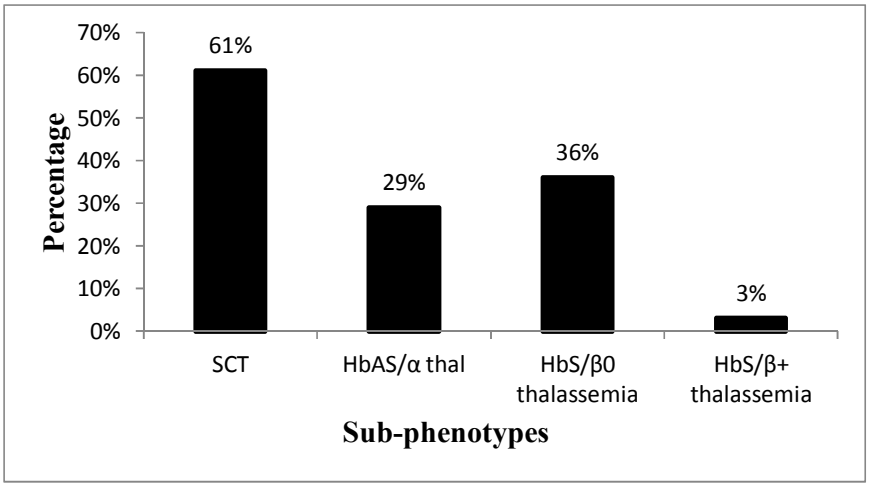
Table 2
Age and Hemoglobin fractions in common Hemoglobin variants

Variables	Normals	Thal minor	Thal intermedia	Thal major	SCT	SCD	HbS/β thal
Age(yrs)	23.8	26.0	11.0	12.4	28.0	20.8	18.4
HbA	97.3 ±0.7	93.0±3.1	43.3±10.8	27.2±16.6	60.9±11.1	11.3±4.5	15.1±10.6
HbA2	2.5±0.5	5.2±0.8	5.0±2.2	1.9±1	2.7±0.5	2.1±1.1	5.0±1.3
HbF	0.2±0.3	1.7±2.6	45.3±0.6	70.9±16.7	2.8±4.2	17.9±6.3	15.6±7.6
HbS	0	0	0	0	33.5±9	68.6±7.1	63.8±11.5

Table 3
Characteristics of uncommon Hemoglobin variants

Phenotype	Cases	Age	HbA	HbA2	HbF	HbS	Unknown
HbE heterozygous	1	25	78.9	20	1.1	0	0
HbE hetero or HbE/β thal	1	18	58.2	24	17.5	0	0
HbE homozygous	2	32	6.3	86.7	6.9	0	0
HbS/HPFH	1	25	16.8	2	50.5	30.7	0
HbS/HbD	2	25	12.9	1.9	13.6	29.8	41.7
HbD/β thalassemia	1	36	18.7	5.3	2.4	0	73.6
HbH hetero/Hb Barts	1	34	88.9	0.9	10.2	0	8.1
HbH	2	23	78.3	1.8	0.6	0	18
HPFH heterozygous	3	17	75.4	1.4	23.2	0	0
HPFH hetero or δβ thal	2	24	86.1	2.9	11	0	0
HPFH /β thalassemia	1	9	59.3	7.5	33.2	0	0
δβ trait	1	28	91.7	1.5	6.8	0	0
HbQ	1	21	82.2	2.5	1.3	0	14

Figure 3
Percentage of Sub-phenotypes



HbAS/α-thalassemia had significant higher mean age, HbA and lower HbF, HbS, Hb% and normal RBC indices as compared to SCT. HbS/β^0-thalassemia had significant lower mean age, HbA, HbF, MCV, MCH & higher HbA2, HbS than HbS/β^+-thalassemia (Table.4)

Table 4
Characteristics of Sickle cell variant subphenotypes

Variables	SCT	HbAS/α-thal	HbS/β^0-thal	HbS/β^+-thal
Age	25.8±13.3	32.0±17.9	18.3±8	19.3±13.7
HbA	56.1±9.4	70.9±7.1	14.3±10.2	24.7±13.1
HbA2	2.7±0.5	2.7±0.5	5.2±1.2	2.9±0.8
HbF	2.9±4.8	2.4±2.6	15.4±7.6	18.6±8.2
HbS	38.0±6.0	24.0±6.7	64.7±11.5	53.8±4.6
Hb	10.8±3.0	8.0±1.1	8.8±0.8	8.3
MCV	74.8±11.8	78.6±7.8	71.7±10.6	81.0
MCH	24.5±5.7	25.8±3.9	23.0±3.3	27.0
MCHC	32.7±2.5	33.0±1.3	32.2±0.2	33.8

DISCUSSION

The incidence of hemoglobin variants in our study differs in certain ways from various prevalent studies from different geographical locations reported from India[6-11] understandably so. Majority of them reported predominantly high prevalence of SCT, followed by SCD, HbS/β−thalassemia, Thalassemia minor, Thalassemia major, and lastly Thalassemia intermedia. Interestingly our patient group had a high prevalence of SCD and also the mean age was 20.8 years. SCD is a serious chronic hemolytic anemia, first manifested in childhood and often fatal before the age of 30 years. A study conducted in a tertiary care hospital in West Bengal by Jain BB et al, the prevalence was only 0.54%[10]. In our cases, most frequent clinical presentation were anemia, hemolytic jaundice, splenomegaly, Avascular necrosis of hip, polyarthralgia, cholelithiasis, postpartum CSVT and stroke. Homozygous patients present with more severe medical conditions and reduced life expectancy than heterozygous individuals who generally are asymptomatic. Eventually, this life-threatening disease displays a complex etiology owing to heterogeneous phenotypes and clinical outcomes, subsequently affecting the management of the patients. Menaa F[12] mentions in his paper that from the general population, twin and familial aggregation studies as well as genome-wide association studies, mostly in pediatric populations with ischemic stroke, showed that the risk of stroke has a substantial genetic component. So, this

high incidence in our patients is in keeping with the natural evolution of the disease per se. In the developed world, the typical patient with SCD has moderately severe anaemia, leads a relatively normal life interrupted by 'crises' as a result of vaso-occlusion, and has a life expectancy of over 45 years[13]. In the sub phenotype of SCT, 30% (n=29) patients had HbS levels less than 24% indicating the presence of α thalassemia with loss of two α-genes. Prevalence of this sub phenotype seems to be higher in our patient group. The mean age also was higher in this group (32 yrs) i.e. late clinical manifestations indicating the protective effect of α-thalassemia i.e. greater affinity of α-globin chains for β^A-globin chains[14]. These cases were referred to us for variant analysis because of chronic anemia and generalised weakness.

Overall gender-wise prevalence of hemoglobinopathies among our study population was higher in males but with no difference in the mean age between the genders. However we could not find explanation for this in any published study. In a study conducted by Jain B.B. et al[10], also had male preponderance (32.04%) and observed that 54.15% were in the age group of 13-36 years followed by 39.88% in 0-12 years. Thalassemia minor cases had mild increase in HbA2 (5.2%), HbF (1.7%), mean Hb of 9.1gm% and normal RBC indices. These patients presented with anemia and mild jaundice. This is in concurrence with the study conducted by Mazza U et al[15]. Occurrence of HbS/β-thalassemia was more in males. Based on the hemoglobin fractions we differentiated HbS/β-thalassemia into HbS/β^+ and HbS/β^0. Clinically HbS/β^+-thalassemia resembles SCT and HbS/β^0-thalassemia resembles SCD[16,17]. HbS/β^0 had significant lower mean age and also the levels of HbA, HbF, MCV, MCH were lower and higher HbA2, HbS than HbS/β^+. We had 3 cases of HbS/β^+ who presented with mild jaundice and splenomegaly unlike the patients with HbS/β^0 who had recurrent jaundice, joint pains and AVN of hip joint. Thalassemia major was the least prevalent in our study group, 1.6% (n=11) but significantly younger age group (mean=12.4yrs) and presented with splenomegaly, jaundice and severe anemia (mean=3.5 gm%). The significant difference between males and females in the mean levels of HbA, & HbF indicates the severity of the disease in the males. Our study closely resembles the study conducted by Bikash Mondal[18]. They also observed a gender associated risk for β-thalassemia of 23.26% and 5.32% for males vs. 15.07% and 1.67% for females in respect of heterozygous and homozygous status. The reason for this male gender susceptibility for abnormal β-thalassemia is unknown. In 2 cases of Thalassemia intermedia in our study, the male patient was 2 yrs old and female patient was 20 yrs old, with Hb of 7 gms% who never required transfusion. In a retrospective study, referred by Clara Camaschella[19], out of 165 Thalassemia intermedia patients of Italian origin, 95% were diagnosed after 2 yrs of age[20]. Different mechanisms are responsible for β-chain production. One mechanism is the preferential survival of F-cells, which operates when β-chain synthesis is absent or very low[21,22]. A second is an inheritable ability to produce more HbF per cell or to increase the number of F cells [22,23].

(Table 3) Though HbE disease is the most prevalent hemoglobin variant worldwide[24], we had only two cases, clinically resembling thalassemia minor. The mean HbA2 was 87.5%. One case of compound heterozygous for HbS/HPFH was a female, aged 25yrs. The clinical course is benign, and vaso-occlusive complications are rare because of the inhibition of sickling by elevated HbF. Two male cases of compound heterozygous for HbS/HbD had an unknown peak (42.5%, 40.9%) in 3.8 min window. The clinical manifestations resemble mild SCD. One male case compound heterozygous for HbD/β thalassemia had mild anemia aged 36 yrs, had increased conjugated bilirubin, microcytosis, phenotype similar to Thalassemia minor. Two males and one female were HPFH Heterozygous, with age range of 11- 20 yrs. They had increased HbF (23.2%) and reduced HbA2 (1.4%). This is a heterogeneous group of conditions having persistent HbF production in adult life in the absence of major hematological abnormalities. Although of little clinical importance, it may modify the phenotype of the β-hemoglobinopathies. The association of heterocellular HPFH with severe homozygous

β-thalassemia is also a mild condition, in spite of 100% HbF[25]. Among the general population approximately 10% of people show high levels of F cells and increased levels of HbF (up to 3%)[23,26,28]. The interaction of heterocellular HPFH with homozygous β-thalassemia results in an improvement of the phenotype[27]. However, the genetics of heterocellular HPFH is quite complex. Certainly some types are related to genes not linked to the β-cluster. There is evidence suggesting an X-linked inheritance[26,28,29]. Recently, a major candidate gene for heterocellular HPFH was identified[31]and mapped in a large Indian family on the long arm of chromosome 6[31]. HbQ case was a 21yr old pregnant female from thalassemic belt in AP, came for the evaluation of cyanosis. Chromatogram showed an unknown band in 4.45min window (14%). This is α gene mutation and in heterozygotes it is clinically silent. So far there are three reports of HbQ in India. The cases in our study group are multiethnic representing wide population, it is indicative of the high morbidity, vulnerability and disease evolution of abnormal hemoglobins in and around the state of Andhra Pradesh. Moreover, all major hemoglobinopathies prevalent all over India have been encountered in our patient population showing thereby the population admixture in this part of India[32].

CONCLUSION

This study provides for the first time the biochemical characteristics of abnormal hemoglobins seen on HPLC method in the evolution of the disease and the clinical presentation of the patients seeking medical help in a tertiary care hospital. Correct diagnosis and understanding of the clinical phenotype are important; otherwise it causes anxiety and mishandling leading to unnecessary investigations of the patients. Familiarity of the physicians with the complexity of the problems will help in the management of these chronic diseases and render enhanced quality of life.

LIMITATIONS

We were unable to confirm the diagnosis by DNA analysis and family studies. As it is a retrospective study some data may be missing.

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