

HEMOGLOBINOPATHIES AMONG FIVE MAJOR ETHNIC GROUPS IN KARACHI, PAKISTAN

Rubina Ghani¹, Mehdi A Manji² and Nikhat Ahmed¹

¹Department of Biochemistry, University of Karachi, Karachi; ²Pathology Laboratories, Shahrah-e-Quaideen, Karachi, Pakistan

Abstract. A brief survey of abnormal hemoglobin variants among the major ethnic groups of Karachi was conducted; 202,600 subjects were studied. Patients with low hemoglobin (Hb), low mean cell volume (MCV) and mean cell hemoglobin (MCH) including anemia, microcytosis, hypochromic hemolysis and target cells, were referred for the identification of hemoglobinopathy by molecular methods. Population screening showed that 60% had iron-deficiency anemia and 40% had hemolytic anemia, of which 20.6% was due to β -thalassemia major, 13% β -thalassemia trait, 5.1% sickle cell disease, 0.76% hemoglobin D Punjab (HbD Punjab), 0.32% hemoglobin C (HbC), and 0.22% hereditary persistence of fetal hemoglobin (HPFH).

INTRODUCTION

The hemoglobinopathies are a diverse group of inherited recessive disorders that include thalassemia and sickle cell disease (Old, 1996). The incidence of β -thalassemia is reportedly high in endemic regions such as the Mediterranean, Africa, Southeast Asia, Southern China, India, and Pakistan. In these regions, β -thalassemia is associated with a hypochromic, hemolytic anemia that sporadically affects most ethnic groups (Najdecki *et al*, 1998; Phadke, 1995).

Migration, illiteracy, nutritional deficiency, and intermarriage among different ethnic groups produce variations of and increases in the prevalence of thalassemia and other abnormal hemoglobins. Different ethnic groups present a range of medical problems including an array of genetic disorders of the red blood cell (RBC). The major disorders are the β -thalassemia syndromes, sickle cell disease, hemoglobin D Punjab (HbD Punjab), and hemoglobin C (HbC) disorder. Other hemolytic anemias, which have multiple RBC abnormalities that are not associated with clinical problems, may be distin-

guished from iron-deficiency anemia (Glader and Look, 1996).

The present study was carried out to determine the prevalence of abnormal hemoglobin variants in the population of Karachi, Pakistan: some 10 million people drawn from five major ethnic groups (Mohajirs, Sindhis, Balochis, Punjabis, and Pathans). Owing to increasing urbanization, ethnic groups from all parts of the country have migrated to and settled in Karachi. This has led to an increase in the transmission of common inherited disorders.

MATERIALS AND METHODS

A total number of 202,600 blood samples were collected from different areas of Karachi; five ethnic groups were represented. Information regarding socio-economic status, nutritional status, literacy, and local traditions was obtained with a survey questionnaire. Hematological screening consisted of a complete blood count (CBC) and estimations of the mean cell volume (MCV) and mean cell hemoglobin (MCH): these tests were conducted using standard procedures for all subjects (Mach-Pascual *et al*, 1996) who were suspected of having a disorder of globin chain synthesis. Abnormal

Correspondence: Nikhat Ahmed, Department of Biochemistry, University of Karachi, Karachi 75270, Pakistan.

hemoglobin variants were identified by cellulose acetate (pH 8.6) and citrate agar (pH 6.2) electrophoresis (Titan III, Helena Laboratories, Texas, USA). Fetal hemoglobin was quantified by the alkali denaturation technique (ICSH, 1979) and Hb A₂ was quantified by cellulose acetate electrophoresis followed by microcolumn chromatography (ICSH, 1978; BCSH, 1998).

DNA was isolated from the peripheral leukocytes of β -thalassemia (major) samples by the salting-out technique (Miller *et al*, 1988). The Amplification Refractory Mutation System (ARMS) was used for the identification of common mutations (Varawalla *et al*, 1991a, b). Table 1 shows the reaction primers used for amplification.

A 25 μ l reaction volume was amplified using a DNA thermal cycler (Perkin Elmer Cetus N808-0009). The amplified products were detected by ethidium bromide stain after electrophoresis on 3% agarose gel (Sigma, A-6013) (Old *et al*, 1990; Ahmed *et al*, 1996).

RESULTS

This study provides comprehensive data on hemoglobin disorders prevalent in Karachi. The map of Karachi (Fig 1) shows the distribution of hemoglobin abnormalities in different areas; Fig 2 shows the percentage of hemoglobinopathies and thalassemia occurring



A.
Iron deficiency anemia,
Thalassemia major,
Thalassemia minor
(Mohajir)

D.
Thalassemia minor, Hb D Punjab
trait, Iron deficiency anemia
(Punjabi, Mohajir)

B.
Sickle cell disease, Hb D
Punjab with Hb S, Hb C
(Baluchi)

E.
Thalassemia minor, Iron
deficiency anemia, Hb D
Punjab trait, Hb C
(Punjabi, Mohajir)

C.
Iron deficiency anemia,
Hb D Punjab trait, Sickle
cell trait, Hb C, Thalassemia
major and minor.
(Pathans, Punjabi, Mohajir)

F.
Thalassemia minor, Iron
deficiency anemia, Hb D
Punjab trait.
(Punjabi, Mohajir)

Fig 1—The geographical distribution of hemoglobinopathies and thalassemia in different localities of Karachi.

Table 1
Primers used for the detection of β -thalassemia mutations by ARMS.

Mutation	Oligonucleotide sequence
IVS I nt 5-n	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAC
IVS I nt 5-m	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG
Fr 8/9-n	CCT TGC CCC ACA GGG CAG TAA CGG CAC ACT
Fr 8/9-m	CCT TGC CCC ACA GGG CAG TAA CGG CAC ACC
Fr 41/42-n	GAG TGG ACA GAT CCC CAA AGG ACT CAA AGA
Fr 41/42-m	GAG TGG ACA GAT CCC CAA AGG ACT CAA CCT
IVS 1 nt 1-n	GAT GAA GTT GGT GGT GAG GCC CTG GGT AGG
IVS 1 nt 1-m	TTA AAC CTG TCT TGT AAC CTT GAT ACG AAA

Table 2
Laboratory features of RBC disorders of an ethnic sub-population of Karachi.

Disorders	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb A (%)	Hb A ₂ (%)	Hb F (%)	Other Hb variants (%)
β -thalassemia major	↓↓	↓↓	↓↓	0	N	↑↑	
β -thalassemia minor	↓	↓	↓	↓	↑(4-7%)	↑(5-10%)	
Sickle cell anemia	↓↓	↓↓	↓↓	0	N	↑(10-20%)	Hb S(70-80%)
Sickle cell β -thalassemia	↓	↓	↓	10-20	↑(4-7%)	↑(25-40%)	Hb S(40-60%)
Sickle cell trait	N	N	N	↓	N	0	Hb S(25-50%)
Hb C disease	slightly ↓	N	N	0	N	0	Hb C(90-95%)
Hb D Punjab	slightly ↓	N	N	40-50	N	10-25	Hb D(25-50%)

N = Normal; 0 = absent; ↑ = moderate increase; ↓ = moderate decrease, ↓↓ = severely decreased; ↑↑ = severely increased.

in different regions of Karachi. The major risk factors included migration and intermarriage, as well as economic, social, cultural, and educational background. The people of the Kharadar, Lyari, Korangi, and Malir localities of Karachi had a low socio-economic status.

Hematological disorders in Pakistan are a major public health problem: they include β -thalassemia (major and minor), sickle cell disease, HbC, HbD Punjab, and the hereditary persistence of fetal hemoglobin (HPFH); in the

study population, these diseases had prevalences of 20.6%, 13%, 5.10%, 0.76%, 0.32%, 0.22%, and 60% respectively (Fig 3).

Abnormalities of mean cell hemoglobin (MCH), mean cell volume (MCV), hemoglobin concentration, and the presence of abnormal hemoglobin variants, were found in all the samples (Table 2). The reduced MCV, MCH, altered Hb A₂ %, and increased Hb F % are the main features of hemoglobin disorders. RBC abnormality may be proportional to the

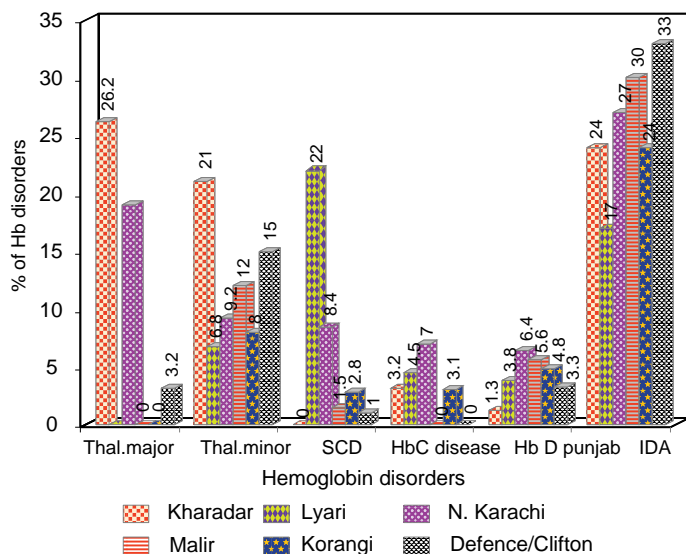


Fig 2–The percentage distribution of hemoglobinopathies and thalassemia in different areas of Karachi.

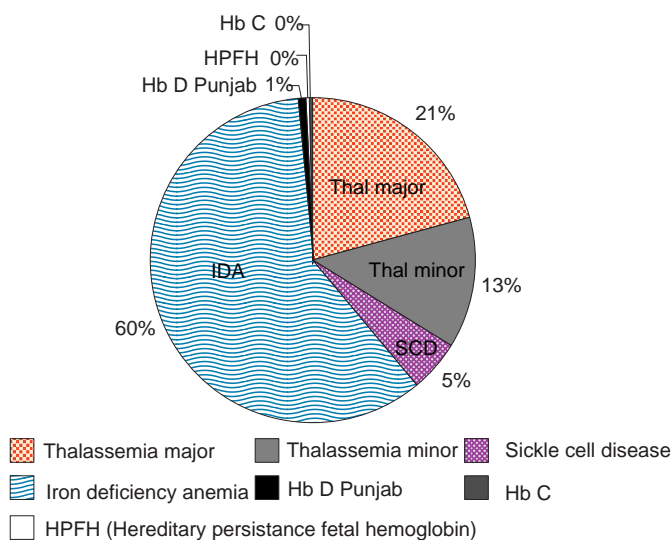


Fig 3–The prevalence of common hematological disorders found in the ethnic population (202,600), of Karachi; 6% of the population had one or more combination of these disorders.

number of deletions in the β -globin gene. The percentage of IVS-1 nt 5 (G→C), IVS-1 nt 1(G→T), codons 41/42 (del TCTT), codons 8/9 (insert G) and 619 bp deletion at the 3' end of the gene in common β -thalassemia mutations are given in Table 3.

The diagnosis of the common forms of α - and β -thalassemia was only possible with genotype-phenotype interactions and was characterized accordingly. Genotype-phenotype distribution was studied to determine the organization of β -globin gene (Table 4).

DISCUSSION

The prevalence of hemoglobinopathies in the ethnic population of Karachi was determined. β -thalassemia is prevalent in the malarious regions of the world, including Pakistan (Fucharoen and Winichagoon, 1997). The average rate of β -thalassemia carriage in Pakistan is 5%; the ethnic groups of Karachi have a rate of 1-2%. β -thalassemia and microcytic anemia are the major health problems faced by the ethnic population of Karachi.

Karachi is divided into districts: the western districts include Kharadar; Lyari, Baldia, North Karachi, and North Nazimabad are northern districts; the southern districts include Clifton and Defence; Korangi and Malir are eastern districts (Fig 1). Inadequate health facilities cater to the large number of sick children. An alarmingly high incidence of β -thalassemia observed in the ethnic groups was substantiated by the information obtained by the survey questionnaire. A positive correlation between the increase in the prevalence of β -thalassemia and the increasing tendency for intermarriage

within ethnic groups was found. The families and parents of patients are generally unaware of or fail to understand hemoglobinopathies: if these disorders are to be addressed, it is essential to adopt a community-based approach. It has been reported that 49% of thalassemic

Table 3
Some common β -thalassemia mutations in major ethnic groups in Karachi, Pakistan.

Mutation	Ethnic groups				
	Punjabis (%)	Pathans (%)	Sindhis (%)	Baluchi (%)	Mohajirs (%)
IVS 1 n 5	22	20	10	15	39
619 bp del	24	50	4	14	3
IVS 1 n 1	13	19	6	10	5
frameshift 8/9	3	6	30	8	9
frameshift 41/42	2	3	10	5	1

Table 4
Organization of β globin gene.

Syndrome	Genotype	Phenotype
β -Thalassemia major	$\alpha\alpha/\beta^0/\beta^0$, β^0/β^+ , β^+/β^+	Severe hemolytic anemia frequent blood transfusion mostly Hb F and Hb A ₂ with variable Hb A.
β -Thalassemia trait	$\alpha\alpha/\beta^0/\beta$ or β^+/β	Mild anemia, mostly Hb A, increased Hb A ₂ , rarely present Hb F.
$\delta\beta$ -Thalassemia trait	$\alpha\alpha/\delta\beta^0/\beta$	Mild anemia, with normal or decreased Hb A ₂ .
Sickle cell anemia	$\alpha\alpha/\beta^S\beta^S$	Severe hemolytic anemia, frequent blood transfusion, mostly Hb S, slightly increased Hb F with normal HbA ₂ .
Sickle β -thalassemia	$\alpha\alpha/\beta^S\beta^{\text{thal}}$	Hemolytic anemia, blood transfusion but rarely, mostly Hb F, and decreased Hb S, normal or increased Hb A ₂ , decreased Hb A.
Hb C disease	$\alpha\alpha/\beta\beta^C$	Mild anemia, moderate amount target cells, normal Hb A ₂
Hb D Punjab	$\alpha\alpha/\beta\beta^D$	Mild anemia, normal Hb A ₂ , decreased amount of Hb A and Hb D present at Hb S.

families show parental consanguinity. Mutation analysis has determined the relationship of mutation pattern and consanguinity and has shown that couples have an 80% chance of a common mutation (Ahmed, *et al*, 1996).

The IVS-1 nt 5(G \rightarrow C) mutation is common among Mohajirs (39%) and Punjabis (22%)

(Table 3). The second commonest mutation found by our study was 619bp del (Pathans 50%), which is different to the prevalence of this mutation that was reported by Old *et al* (1990) and Ahmed *et al* (1996). Previous studies of Pakistani immigrants to the United Kingdom have shown that the 619 bp deletions are present in 56% of Sindhi and 13.9% of native

Sindhi subjects (Varawalla, 1991a,b; Ahmed *et al.*, 1996). The high prevalence of the 619 bp deletion was observed in selected groups of patients, although not a true Sindhi population (Varawalla, 1991a); however, Verma *et al.* (1997) reported that frequency of 619 bp deletions was 33.3%. The IVS 1nt1(G→C) was present in Pathans (19%); this mutation was reported previously by Verma *et al.* (1997), who found that 26% of immigrants from Pakistan had IVS 1 nt 1 (G→T).

Microcytosis was also a common finding during blood examination; microcytosis was defined as a mean corpuscular volume (MCV of < 82 fl) (Table 2) and is useful in the differential diagnosis of microcytic and macrocytic anemia. MCV and MCHC also provide a useful tool for characterizing red blood cells in patients with anemia (Schaefer and Schaefer, 1999). Microcytosis is of importance in the laboratory diagnosis of hemoglobinopathies and is of especial relevance in the diagnosis of abnormal globin chain synthesis.

Genotype-phenotype correlation plays an important role in the management of thalassemia and hemoglobinopathies. In the population survey, genotype-phenotype interaction explains, to a certain extent, the heterogeneity of severity of the clinical phenotypes that were reported among patients from different regions of Karachi. The interaction of the genotype with clinical and hematological phenotypes was characterized in order to assess the types of abnormal hemoglobin disorders. The characteristic hematological changes of β -thalassemia minor (carrier state) and sickle cell β -thalassemia may be modified by the genetic factors. The most common atypical abnormal hemoglobin variants with corresponding genotype-phenotype are summarized in Table 4. These data tell us that:

The identification of silent carriers with normal MCV, MCH and Hb A₂. If suspected on the basis of borderline Hb A₂ levels, identification of silent carriers may be made using globin chain synthesis analysis (sometimes slightly unbalanced) or characterization of DNA mutations;

In populations in which β -thalassemia and other common abnormal hemoglobin variants are present, Hb A₂ determination may be carried out in order to identify double heterozygous for ($\alpha\alpha/\beta^0/\beta^0$ ---genotype) β -thalassemia;

Globin chain synthesis and /or globin gene analysis and /or family studies are necessary to differentiate double heterozygotes and carriers of some mild β -gene mutations.

We concluded that all the regions either have, or have recently had, endemic malaria. Hemoglobinopathies occur commonly in such regions. Therefore the occurrence of hemoglobinopathies in non-malarious areas suggests a genetic drift due to migration. The migration of ethnic groups from all parts of Pakistan is ongoing; many migrants settle in Karachi. The major risk factors include migration and intermarriage as well as economic, social, cultural, and educational background. In addition, inadequate health facilities also add to the inability to treat large numbers of sick children. The families and parents suffer from ignorance and misapprehension; therefore, to enable control of hemoglobinopathies and thalassemia, it is essential to approach this problem at the community level. This requires good technical support and proper education.

Extreme heterogeneity in the clinical and hematological phenotypes are associated with genotype interaction.

ACKNOWLEDGEMENTS

We thank Dr Furqan Hasan and all the staff of the National Institute of Child Health Hospital (NICH) for their support and assistance. We would also like to acknowledge the cooperation of the clinics and pathology laboratories that helped with sample collection.

REFERENCES

- Ahmed S, Petrous M, Saleem M. Molecular genetics of beta thalassemia in Pakistan: a basis for prenatal diagnosis. *Br J Haematol* 1996; 94: 476-82.

- British Committee for Standards in Haematology, Working Party of the General Haematology Task Force. The Laboratory Diagnosis of Haemoglobinopathies. *Br J Haematol* 1998; 101: 783-92.
- Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia. Molecular biology and clinical medicine. *Hemoglobin* 1997; 21: 299-319.
- Glader BE, Look KA. Hematologic disorders in children from Southeast Asia. *Pediatr Hematol* 1996; 43: 665-81.
- International Committee for Standardization in Haematology (ICSH). Recommendations for selected methods for quantitative estimation of Hb A₂ and for Hb A₂ reference preparation. *Br J Haematol* 1978; 38: 573-8.
- International Committee for Standardization in Haematology (ISCH). Recommendation for fetal hemoglobin preparation and fetal hemoglobin determination by alkali denaturation method. *Br J Haematol* 1979; 42: 133-6.
- Mach-Pascual S, Darbellay R, Pilotto PA, Beris P. Investigation of microcytosis: a comprehensive approach. *Eur J Haematol* 1996; 57: 54-61.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
- Najdecki R, Georgiou I, Lolis D. The thalassemia syndromes and pregnancy, molecular basis, clinical aspects, prenatal diagnosis. *Ginekol Pol* 1998; 69: 664-8.
- Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of β -thalassemia: studies in Indian and Cypriot populations in UK. *Lancet* 1990; 336: 834-7.
- Old J. Hemoglobinopathies. *Prenat Diagn* 1996; 16: 1181-6.
- Phadke MA. Prevention of thalassemia. *Southeast Asian J Trop Med Public Health* 1995; 26 (suppl 1): 261-5.
- Schaefer RM, Schaefer L. Hypochromic red cells and reticulocytes. *Kidney Int* 1999; 55, (suppl 69): S-44-S-48.
- Varawalla NY, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of β -thalassemia mutations on the Indian subcontinent: the basis for prenatal diagnosis. *Br J Haematol* 1991a; 78: 242-7.
- Varawalla NY, Old JM, Weatherall DJ. Rare β -thalassemia mutations in Asian Indians. *Br J Haematol* 1991b; 79: 640-4.
- Verma IC, Sexena R, Thomas E, Jani PK. Regional distribution of beta-thalassemia mutations in India. *Hum Genet* 1997; 100: 109-13.