Haemoglobinopathies – thalassaemias and abnormal haemoglobins in eastern Uttar Pradesh and adjoining districts of neighbouring states

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The haemoglobinopathies – thalassaemias and abnormal haemoglobins – constitute a major burden of genetic diseases in India. Our study, based on index cases from 120 families detected between May 1999 and May 2003, highlights the ethnic distribution of haemoglobinopathies in regions in and around Varanasi comprising 8–10 districts of eastern Uttar Pradesh and

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adjoining districts of Bihar, Jharkhand, Chhattisgarh and Madhya Pradesh. Homozygous and heterozygous *b*-thalassaemia was the most common (66.9%), with thalassaemic haemoglobinopathies HbE-*b*-thalassaemia (15.9%) and HbS-*b*-thalasseamia (7.8%) contributing to almost a quarter of the cases. Along with HbSS disease (4.3%), the results indicate a confluence of *b*-thalassaemia, HbS and HbE in this region. IVS1-5 nt was the most common mutation in the few carriers analysed for mutation detection. The significance of the study lies in the demonstration of wide prevalence of *b*-thalassaemia across all castes and communities of this region, with migrant population groups of Sindhis and Punjabis comprising only 5.8% of the index cases. Also, HbE seems to have a much higher presence in this region than so far believed and HbS has a significant presence in general castes as well.

THE inherited disorders of haemoglobin – thalassaemias and haemoglobinopathies – constitute a major burden of genetic disorders in India. Reports from different parts of the country have enabled mapping of ethnic distribution and prevalence of thalassaemias and the three common symptomatic abnormal haemoglobins S, D and E[1–3]. In the studies from north India, major groups of thalassaemics from Uttar Pradesh are the migrant ethnic populations of Punjab and Sindh origin[4–7]. The presence of HbE in Uttar Pradesh (UP) has been detected by way of symptomatic cases of HbE *b*-thalassaemia. In the cases recorded between 1987 and 1995, Agarwal *et al.*[8] reported 21 families with HbE-*b*-thalassaemia. Sporadic cases have been mentioned in a few other reports[9–11]. According to Balgir[2], the average frequency of HbS gene in UP is around 7.2%, mostly in tribal and scheduled caste populations, but permeating into general castes as well. Nearly 28 mutations in *b*-globin gene have so far been recorded in the Indian subcontinent, among which five (IVS1-5 (G → C), –619 bp del, IVS1-1 (G → T), CD8/9 (+G), CD41/42 (-CTTT)) account for more than 90% of the cases[4].

Eastern UP and adjoining regions of Bihar, Madhya Pradesh (MP), Chhattisgarh and Jharkhand are poorly represented in the studies from northern India. Varanasi serves as a medical referral centre for this region. In this study, data based on 120 index cases from unrelated families detected over a period of four years from May 1999 to May 2003, at the only referral laboratory for thalassaemia in Varanasi, are presented. The present study documents considerable presence of thalassaemias, haemoglobinopathies and thalassaemic haemoglobinopathies in the native populations of these regions, largely independent of caste.

All the index cases reported here came from unrelated families residing in this region. These cases were referred to our laboratory from the hospital in Banaras Hindu University (BHU) and other private clinics between May 1999 and May 2003. The partial map of India (Figure 1) illustrates the geographical regions from where the cases were referred. Two cases, initially detected elsewhere and reported to our laboratory for follow-up, have also been included here. Mutational analysis was carried out in the Cytogenetics Laboratory, Department of Zoology, BHU.

Diagnosis of each case was based on haematological investigations which included complete blood counts on semiautomated blood cell counter (Danam HC 5710), peripheral smear examination, reticulocyte count and HbH inclusions. Electrophoresis on cellulose acetate at pH 8.6 allowed resolution of different haemoglobins[12]. HbA2 and HbE were estimated by microcolumn chromatography using glycine–cyanide developers, and foetal haemoglobin was estimated by alkali denaturation method of Singer[12]. Wherever required, these tests were supplemented by red-cell osmotic fragility, serum bilirubin and liver function tests, serum iron and iron-binding capacity and sickling test[12]. Diagnosis of HbE-*b*-thalassaemia and HbS-*b*-thalassaemia in all index cases has been confirmed by simultaneous studies in parents, where one parent had *b*-thalassaemia

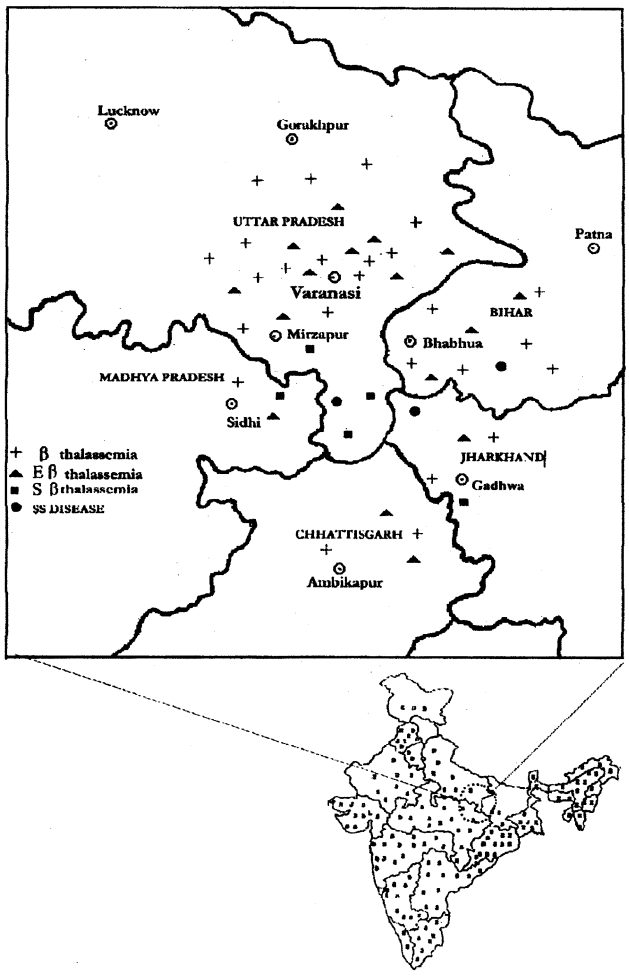

Figure 1. Partial map of India depicting regions of eastern Uttar Pradesh and adjacent States from where samples have been referred. The map of India below indicates the location of this region in the national context. Symbols indicate different haemoglobinopathies represented proportionately.

trait and HbE or HbS trait in the other. The thalassaemic phenotype has been broadly classified into thalassaemia major, intermedia and minor based on standard criteria mentioned[13,14].

For detection of mutation, genomic DNA of suspected carriers, extracted from a small sample of peripheral blood, was PCR-amplified using the standard ARMS strategy of common mutation detection in *b*-globin and HbE (viz. IVS1-1nt G-T, IVS1-5 nt G–C, CD 8/9 +G, CD 41/42 (-TCTT), HbE CD 26 G–A; standard primers obtained from Genei, India). The PCR conditions were as recommended. DNA (100 ng) was subjected to 30 cycles of denaturation, annealing and extension at 94, 58 and 72°C respectively. The reaction was carried out in a programmable thermal cycler (Genius, TECHNE, UK).

Out of 120 cases, 80 (67%) were of *b*-thalassaemia (*b*/*b* = 59; *b*/*A* = 21), 19 (15.9%) of HbE-*b*-thalassaemia, and 9 (7.8%) of HbS-*b*-thalassaemia, all belonging to Varanasi and surrounding regions of eastern UP, neighbouring districts of Bihar, Jharkhand, MP and Chhattisgarh. In addition, there were five cases of homozygous sickle cell disease, two of HbH disease and two each of HbD trait and HbS trait. Single case of HbE trait was also detected. Regional distribution is depicted in Figure 1.

Distribution of different haemoglobinopathies presently examined is given in Table 1. Patients with thalassaemic phenotype have been broadly classified into thalassaemia major (transfusion-dependent disease), intermedia (largely transfusion-independent growth with transfusional support required during intercurrent illnesses/crises leading to sudden drop in Hb levels) and minor, i.e. asymptomatic cases. Among the 80 cases of *b*-thalassaemia, 59 (49%) were of homozygous *b*-thalassaemia (45 presented as thalassaemia major and 14 as thalassaemia intermedia), 21 (17%) of heterozygous *b*-thalassaemia (MCV < 80 fl, normal serum iron, HbA2 > 3.5%, HbF < 5%), of which two were asymptomatic (thalassaemia minor) and 19 were assigned to thalassaemia intermedia phenotype. All but one of these 19 cases presented to us in adulthood with signs and symptoms severe enough to require medical support and pertaining to chronic refractory haemolytic anaemia. The clinico-haematologic data of these cases are presented in Table 2. There were 19 cases of HbE-*b*-thalassaemia and 9 (23.3%) of HbS-*b*-thalassaemia. Among the HbE-*b* cases, the thalassaemia major and intermedia phenotypes were almost in equal proportions, 9 and 10 respectively. Five of these thalassaemia intermedia cases were presented to us between 12 and 32 years of age with moderate to massive splenomegaly, chronic anaemia with Hb levels ranging from 4.2 to 6.4 g/dl, mild icterus and mild haemolytic facies. The remaining four, presented between 4 and 5 years of age, were transfusion-independent with mild to moderate splenomegaly. One case was diagnosed at 2 years of age. The HbS-*b*-thalassaemia patients had more severe disease with lower Hb levels and systemic involvement than their counterparts having homozygous sickle cell disease (HbSS; five cases).

Broad ethnic classification of the cases (Table 3) revealed that more than 80% cases were native Hindus of this region; 50% belonging to Brahmin, Thakur, Bania, Marwari and Kayasth communities, and 34.1% were mostly Yadavas, Kurmis, Kharwars, Vishwakarmas and scheduled castes. Punjabis and Sindhis contributed only 5.8% of the cases. Thirteen (10.8%) cases were from Muslim families among which ten were of homozygous-*b*-thalassaemia, two of HbE-*b*-thalassaemia and one of HbSS disease (from Bihar). Some cases assigned to 'unknown' group, were all Hindus but their specific caste was not known.

All but one of the five homozygous sickle cell disease (HbSS) index cases belonged to scheduled castes and tribes. The lone Brahmin family belonged to Sonebhadra region. However, of the nine index cases of HbS-*b*-thalassaemia, five were Kshatriya and Brahmin. Only two cases of HbD were detected of which one was accidentally detected in an asymptomatic 32-year-old Punjabi male. The other was a four-year-old Sindhi anaemic boy, whose mother was a carrier for HbD trait. However, his father did not show any abnormal haemoglobin or *b*-thalassaemia trait by the

Table 1. Haemoglobinopathies in Varanasi region and their clinical phenotypes

Clinical phenotype	Thalassaemia major		Thalassaemia intermedia		Thalassaemia minor		Sickle cell disease		Asymptomatic		Total	
Haemoglobinopathies	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
b/*b*-thalassaemia	45	81.8	14	30.4	–		–		–		59	49.1
b/*A*-thalasseamia	–		19	41.3	2	100	–		–		21	17.5
HbE-*b*-thalassaemia	9	16.4	10	21.7	–		–		–		19	15.9
HbE trait			1	2.2	–		–		–		1	0.8
HbS/S disease	–		–		–		5	35.7	–		5	4.3
HbS-*b*-thalassaemia							9	64.3			9	7.8
HbS trait			–		–		–		2	50.0	2	1.7
HbH disease			2	4.3	–		–		–		2	1.7
HbD trait					–		–		2	50.0	2	1.7
Total	55	45.8	46	38.4	2	1.7	14	11.6	4	3.3	120	100

Table 2. Clinico-haematologic data of heterozygous *b*-thalassaemia cases – thalassaemia intermedia

Age/sex	Hb (g/dl)	MCV/MCH/ RDW	RBC morphology	Hb electrophoresis	HbA2/HbF		Important clinical features
25 yr/F	6.7	69.0/22.5/15.9	Microcytic hypochromic	A + A2	4.1	0.0	Aggravation of anaemia during second trimester of pregnancy – maintained on transfusion till delivery.
48 yr/M	7.9 `	58.6/20.5/16.4	Anisocy++, micr++ hypo++++	A + A2	6.6	1.3	Persistent weakness and intermittent icterus aggravating during intercurrent illness. Mild hepatosplenomegaly.
32 yr/M	7.6	62.3/22.5/21.0.	Thalassaemic	A + F + A2	6.7	1.5y	Persistent weakness and imtermittent jaundice aggravating after intercurrent illness. Spleen palable.
12 yr/F	7.4	–	Thalassaemic	A + A2	5.4	0.8	Persistent weakness.
26 yr/F	6.4	–	Thalassaemic	A + F + A2	6.1	3.2	Severe refractory anaemia during 24–28 weeks of pregnancy – maintained on regular transfusion till delivery.
25 yr/F	8.4	63.2/21.9/16.0	Microcytic hypochromic	A + A2	4.8	0.0	Mild weakness aggravating after illness.
13 month/M	8.1	65.3/21.8/16.0	Microcytic hypochromic	A + A2	6.2	0.8	Pt presented with refractory moderate anaemia and mild splenomegaly.
22 yr/M	6.4	–	Thalassaemic	A + F + A2	4.6	2.0	Intermittent jaundice and weakness aggravating during intercurrent illness.
25 yr/M	7.7	64.4/22.5/20.9	Thalassaemic	A + A2	4.3	1.7	Intermittent jaundice and weakness aggravating during intercurrent illness. Palpable spleen.
40 yr/F	7.7	66.1/24.2/15.4	Microcytic hypochromic	A + A2	3.5	0.0	Persistent weakness aggravating after mild intercurrent illness.
25 yr/M	7.1	61.4/19.6/15.2	Thalassaemic	A + F + A2	5.0	2.0	Intermittent jaundice and weakness aggravating during intercurrent illness. Liver 2.0 cm, spleen 2.5 cm.
15 yr/M	10.2	59.5/20.8/15.7	Microcytic hypochromic	A + A2	4.1	0.0	Weakness, mild intermittent jaundice, absence of secondary sexual characteristics
16 yr/M	5.8	64.1/22.3/18.1	Thalassaemic	A + A2	5.8	0.0	Intermittent jaundice and weakness aggravating during intercurrent illness. Spleen 2.0 cm.
15 yr/F	7.3	69.9/24.5/18.0	Thalassaemic	A + A2	4.0	0.0	Increased weakness after mild fever/illness.
30 yr/M	6.1	62.1/21.1/18.5	Thalassaemic	A + F + A2	3.6	6.2	Intermittent jaundice and weakness aggravating during intercurrent illness. Spleen+.
45 yr/M	5.0	–	Thalassaemic	A + A2	4.3	0.9	Presented with breathlessness and weakness
31 yr/M	6.5	58.7/22.1/15.9	Thalassaemic	A + A2	5.2	0.9	Intermittent jaundice and weakness aggravating during intercurrent illness.
18 yr/M	10.0	59.5/19.9/16.7	Thalassaemic	A + F + A2	6.3	2.6	Intermittent jaundice and weakness aggravating during intercurrent illness. Mild hepatosplenomegaly.
45 yr/F	9.2	57.6/20.7/16.9	Microcytic hypochromic	A + A2	7.3	0.0	Persistent weakness with aggravation during fever.

above mentioned methods. Further investigations could not be carried out due to lack of cooperation from the family. Also, there were two cases of *a*-thalassaemia, HbH disease diagnosed on the basis of microcytic hypochromic blood counts, normal HbA2 and serum iron and presence of HbH inclusions, both from lower caste Hindu families, one each from UP and Bihar.

ARMS PCR was performed on 31 individuals to assess the frequency of different common mutations in this region. Six samples came from two families (proband and both parents). In the two families, one proband was homozygous for IVS1-1, while the other was a compound heterozygote for IVS1-1/5. Both these families were of Sindhi origin. Rest of the 25 samples were from carrier parents

Table 3. Ethnic distribution of haemoglobinopathies in Varanasi region

Religion/caste/ ethnic group	Brahmins, Kshatriyas, Banias and Kayasths		Yadavas, Kharwars, Mauryas, Kurmis and SC/STs		Muslims		Punjabis		Sindhis		Unknown		Total	
Haemoglobinopathies	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
b-thalassaemia (*b*/*b* and *b*/*A*)	39	75.0	23	56.1	10	76.9	2	66.7	3	75	3	42.9	80	66.9
HbE-*b*-thalassaemia	6	11.4	8	19.5	2	15.4	0	–	0	–	3	42.9	19	15.9
HbE trait	1	1.9	0	–	0	–	0	–	0	–	0	–	1	0.8
HbS/S disease	1	1.9	3	7.3	1	0.8	0	–	0	–	–	–	5	4.3
HbS-*b*-thalassaemia	4	7.7	4	9.8	0	–	0	–	0	–	1	14.3	9	7.8
HbS trait	1	1.9	1	2.5	0	–	0	–	0	–	0	–	2	1.7
HbH disease	0	–	2	4.9	0	–	0	–	0	–	0	–	2	1.7
HbD trait	0	–	0	–	0	–	1	33.3	1	25	0	–	2	1.7
Total	52	43.3	41	34.1	13	10.8	3	2.5	4	3.3	7	5.8	120	100

or a kin of the proband. Five samples did not yield results. In the rest, the distribution of different alleles was as follows: IVS1 nt 5–15, IVS1 nt 1–3, CD 8/9 + G-1 and CD 41/42 +G-1.

In north India, the incidence of *b*-thalassaemia has been mainly attributed to its high prevalence in the migrant populations of Sindhi and Punjabi origin[4,5,7,8,15]. HbE, the abnormal haemoglobin, shows high incidence in Northeastern States and West Bengal, with a few cases in UP and Bihar[2,8–11]. HbS is reported to be prevalent among tribal and lower castes in MP. Thus different haemoglobinopathies have varied distributions in different geographical regions and ethnic groups. However, there are hardly any reports on their distribution in eastern UP and Bihar. The present study of 120 cases from unrelated families was carried out to unravel the ethnic diversity of thalassaemias and abnormal haemoglobins in these regions. Unlike previous reports, only five of our 80 *b*-thalassaemia cases, belonged to Punjabi and Sindhi families. In contrast, 72 (more than 80%) were native Hindus and Muslims, demonstrating that *b*-thalassaemia is no longer confined to specific ethnic groups; instead it is widely distributed in all castes and communities native to this region (Table 3). Similarly, occurrence of 16.7% cases of HbE in the present group of samples, when viewed together with the reports of Agarwal *et al.*[8] and Dube *et al.*[9], suggests the spread of HbE well beyond West Bengal through Bihar to eastern UP. Also, distribution of HbE-*b*-thalassemia in all castes and communities indicates a much wider prevalence of HbE than hitherto assumed.

The other abnormal haemoglobin, HbS, which is reported to be prevalent in tribal backgrounds of MP, was also found in general castes of Hindus (5/9 cases of HbS-*b*-thalassaemia). All of them belonged to Mirzapur–Sonebhadra region of UP, which is contiguous with the high HbS tribal regions of MP.

The simultaneous presence of *b*-thalassaemia, HbE and HbS in this region appears to contribute significantly to the load of haemoglobinopathies in these populations. Since many of these patients live up to adulthood with active reproductive life, they increase the chance of propagation of both *b*-thalassaemia and abnormal haemoglobin genes. It is not unreasonable to assume that fewer reports of HbE-*b*-thalassaemia from this region could be because of underdiagnosis due to low index of suspicion, prevailing ethnic bias, and absence of diagnostic facilities. Krishnamurthy[16] has also suspected the same for the Northeastern populations.

Though a large number of *b*-thalassaemia-causing mutations are recorded in *b*-globin gene, interestingly, only a few seem to occur in any population. In India, five common mutations (IVS1-1, IVS1-5, –619 bp del, CD 8/9 +G, CD 41/42 (-CTTT)) account for more than 90% of thalassaemia carriers[4]. Among these, CD 8/9, IVS1-5 and –619 bp del occur in around 80% of the cases. However, in the two major studies comprising patients from UP and Bihar[4,5] IVS1-5 was the most common mutation, accounting for more than 50% of the cases followed by CD 41/42 (-TCTT) while three other mutations had low frequencies. Similar results were obtained in an earlier study on cases from Kolkata[17]. Curiously, the uncharacterized mutations in all these studies mostly belonged to UP and Bihar. Mutation IVS1-1 has largely been detected in the migrant Sindhi population. In the present study on a small sample, the majority of carriers are IVS1-5. Six IVS1-1 alleles were characterized in two patients from Sindhi families and their parents. The two cases of IVS1-1 recorded happen to be parents of a *b*-thalassaemic child. However, they remain ethnically uncharacterized. Thus, while IVS1-5 is the common mutation even in these populations, a larger sample is required to be studied to have an estimate of the frequency of other mutations.

As most of the subjects in our study were symptomatic cases referred for investigation of refractory anaemia, the results only reflect the significant presence of *b*-thalassaemia and abnormal haemoglobins E and S in this region

across all castes and communities. Screening of healthy population is required to determine the carrier rates and gene frequencies in this region.

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