Haemoglobin D in a mongoloid non-tribal family: A report from northeast India

Haemoglobin E (HbE) is the most frequently encountered haemoglobin variant among the autochthonous Mongoloid inhabitants of northeastern India. In Assam, besides HbE among the Mongoloid group, sickle cell haemoglobin (HbS) is predominant among the tea-plantation workers. We have documented haemoglobin D (HbD) in an Ahom family belonging to a Mongoloid non-tribal group, in double heterozygous (HbDE) as well as in HbD-heterozygous (HbAD) state from Assam.

In India gene frequency for HbD ($\beta^{121Glu \rightarrow Gln}$) is relatively low, with a tendency to cluster towards the northwestern part of the country[1,2]. Prevalence of HbD in India has been documented with variable gene frequency from Punjab, Gujarat, Jammu & Kashmir, Uttar Pradesh, Maharashtra, Karnataka, Orissa, West Bengal and Goa, either in the homozygous or heterozygous state[1,2]. So far, this abnormality has not been reported from the northeastern region of India. Our search in MEDLER, MEDLINE standard and other relevant published literature failed to reveal any report of HbD from this region of the country.

The most common structurally defective haemoglobin reported among the autochthonous inhabitants of northeastern India, is HbE (refs 3–6). Besides HbE, HbS is also observed among the tea-plantation workers of Assam, a group of tribal people from central, eastern and southern India, indentured by the British to work in tea gardens during early 19th century[7]. We report HbD in a family belonging to Ahom community, a non-tribal Mongoloid group of Assam, both in double heterozygous (HbDE) and heterozygous (HbAD) state.

During our screening programme for haemoglobinopathy, an ongoing programme of our centre, blood sample was obtained from a 22-year-old, apparently healthy Ahom boy (CK). Electrophoresis of the haemoglobin on cellulose acetate at alkaline pH[8] indicated the presence of HbE and HbD/HbS. The sample failed to produce sickling in the presence of sodium dithionite[8], and the qualitative solubility test, indicating absence of HbS[8]. Blood sample analysis on automated haematology cell counter indicated low MCV (63 fl) and a positive result for the naked-eye single-tube osmotic fragility test (NESTROFT)[9]. Zinc protoporphyrine (ZPP) level was normal (53 µmol/mol of heme). Result of the Variant Haemoglobin Testing System using beta thalassaemia short programme also indicated presence of HbE and HbD (D-window – 61.2% with a retention time of 4.24 min). Details of haematological indices and data of Variant Haemoglobin Testing System are incorporated in Table 1 and Figure 1.

Family members of the subject (CK) were contacted for family study. It was informed by the father of the subject (GK) that the last three generations of their family are residing in a village, which is about 12 km from Dibrugarh town. The other members of his clan are residing in a nearby village and as far as he remembers, there is no report of inter-caste marriage either in the family or in their last two generations.

After proper counselling and obtaining an informed written consent, about 5 ml of venous blood from each member of the family was collected in EDTA as an anticoagulant. The samples were immediately transported to the laboratory and on the same day they were subjected to an automated haematology cell counter (Sysmex SF 3000) to determine the haematological indices. ZPP level was also measured by an automated machine (Protoflouro Z, Helana). Electrophoresis of the samples, after preparation of the haemolysates, was performed on cellulose acetate in alkaline pH[8]. Finally, samples were analysed on Variant Haemoglobin Testing System (BioRad, Version 4.74a) according to the protocol of the manufacturer.

Haematological indices of the blood samples reflect lower level of MCV among the family members. Absence of HbS was documented, as the blood samples failed to produce sickling in the presence of sodium dithionite and during qualitative solubility test. Observation of normal level of ZPP (39–69 µmol/mol of heme) among the family members indicates lack of iron deficiency. Electrophoretic pattern and results of Variant Haemoglobin Testing System indicate presence of HbD along with HbE (HbDE) in one of the parents and HbAE in the other. Double heterozygous stage for HbD and HbE (HbDE) was observed in two offspring, while the other two were with HbAD. Retention time for HbD (D-window) in subjects with HbDE and HbAD was between 4.23 and 4.24 min. The reported retention time for HbD-Los Angeles is 4.16 min (ref. 10) against 4.23–4.24 min observed by us. This indicates that the present HbD has a similarity with HbD-Los Angeles. Slight difference of retention time in our study

Table 1. Haematological indices and zinc protoporphyrin status of the family with HbD

		Haematological indices										
Name	Age/ sex	WBC $10^3/\mu l$	RBC $10^6/\mu l$	Hb g/dl	HCT %	MCV fl	MCH pg	MCHC g/dl	RDWs fl	PLT $10^3/\mu l$	MPV fl	ZPP µmol/ mol heme
GK	60/M	6.6	5.54	11.1	35.2	64	20.1	31.6	47.7	113	9.2	64
SK	45/F	5.9	5.46	12.0	37.9	69	22.0	31.8	43.0	211	12.6	60
CK	22/M	5.9	6.98	13.3	43.8	63	19.1	30.4	43.8	75	12.1	53
RK	20/F	6.5	5.34	10.9	34.7	65	20.5	31.4	41.4	107	12.7	69
DK	16/M	5.0	5.46	14.1	43.3	79	25.8	32.6	46.1	85	11.7	39
DK	13/M	4.3	5.61	14.2	44.1	79	25.3	32.2	46.9	146	13.5	40

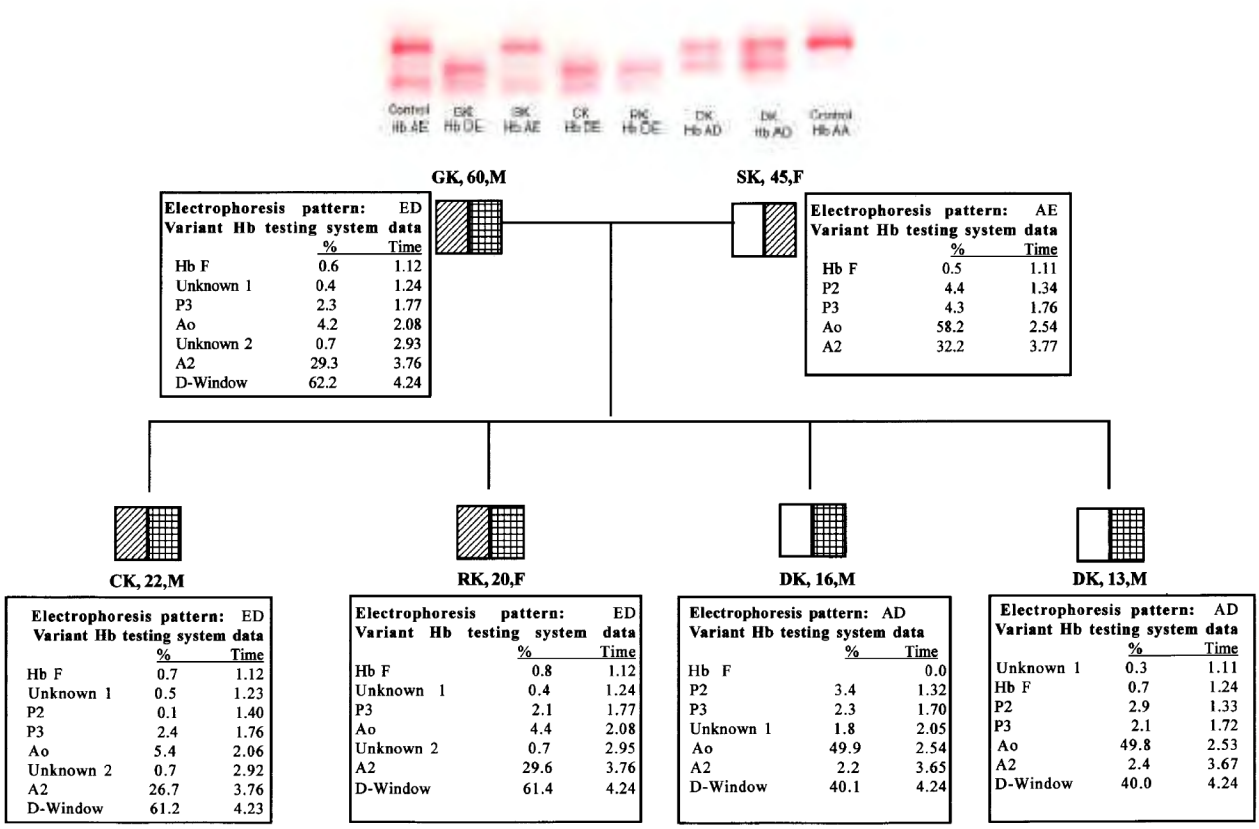

Figure 1. The family tree, electrophoretic pattern and data on Variant Haemoglobin Testing System in subjects with HbD.

may be due to instrumental variation. However, irrespective to their haemoglobin type, HbF level was normal for the entire family, while normal level of HbA_2 was observed for subjects with HbD-heterozygous (details incorporated in Figure 1).

The present finding indicates prevalence of HbD in the family belonging to the Ahom community. Presence of HbDE in one of the parents and HbAE in the other designates co-inheritance of HbE and HbD globin genes by the elder son (CK–22 M) and daughter (RK–20 F). Similarly, the remaining two sons (DK–16 M and DK–13 M) with HbAD indicate inheritance of a normal HbA gene from their mother and HbD gene from their father.

The northeastern region of India is home to people with an assortment of socio-cultural, linguistic and ethnic diversity due to the migration of various races. Documentation regarding migration of the Sikhs, as early as 16th century, to Assam is available and their descendents are presently known as *Asomia Sikhs*[11]. However, other population migrations, known to be prevalent with HbD, from the rest of the country to the northeastern states took place much later. Therefore, the possible reason for detection of HbD in the Ahom community has to be ascertained and requires further exploration.

This is a report documenting HbD, both in heterozygous and in double heterozygous state, from a Mongoloid non-tribal group of Assam. Further characterization of this haemoglobin may provide interesting phenomenon to understand its molecular composition[12].

1. Sharma, A., *Peoples of India – Some Genetic Aspects* (ed. Satyavati, G. V.), Indian Council of Medical Research, New Delhi, 1983, pp. 31–49.
2. Balgir, R. S., *Curr. Sci.*, 2000, **79**, 1536–1547.
3. Deka, R., Gogoi, B. C., Hundrieser, J. and Flatz, G., *Hemoglobin*, 1987, **11**, 531–538.
4. Das, B. M., Chakaravarthy, M. R., Delbruck, H. and Flatz, G., *Humangenetik*, 1971, **12**, 264–266.
5. Flatz, G., Chakravarthy, M. R., Das, B. M. and Delbruck, H., *Hum. Hered.*, 1972, **22**, 323–330.
6. Das, B. M., Deka, R. and Das, R., *J. Indian Anth. Soc.*, 1980, **15**, 153–156.
7. Balgir, R. S. and Sharma, S. K., *Indian J. Hematol.*, 1988, **6**, 1–14.
8. Dacie, J. V. and Lewis, S. M., *Practical Haematology*, Churchill Livingstone, Edinburgh, 1984, 6th edn, pp. 179–199.
9. Kattamis, C. and Efremov, C., *J. Med. Genet.*, 1980, **18**, 266–270.
10. BioRad Variant™, BioRad. Version 3, 270-0259, 41.
11. Medhi, B. K., *Peoples of Northeast India* (ed. Sengupta, S.), Vyas Publishers, New Delhi, 1996, pp. 191–198.
12. Fioretti, G. *et al.*, *Hemoglobin*, 1993, **17**, 9–17.

Received 31 October 2002; revised accepted 6 January 2003

S. K. SHARMA
S. GOGOI
R. DUTTA
J. MAHANTA*

Regional Medical Research Centre,
N.E. Region,
(Indian Council of Medical Research),
Post Box No. 105,
Dibrugarh 786 001, India
**For correspondence.*
e-mail: icmrrcdi@hub.com