Correspondence

Status of HbE variant among Rabha tribe of West Bengal, India

Sir,

Haemoglobin E (HbE), a common structural haemoglobin variant occurs in very high frequency in countries of south-east Asia and in north-east India. Rabha tribe, one of the scheduled tribes of India, is mainly found in Assam and West Bengal. In north Bengal, 70 per cent of Rabhas dwell in Jalpaiguri and Cooch Behar districts. This pilot study was undertaken in a Rabha Basti under the Madarihat police station of the Alipurduar subdivision of Jalpaiguri district, West Bengal, India, to observe and analyze the occurrence of HbE trait among the Rabha tribe.

At Rabha Basti, 286 individuals (234 Rabhas and 52 non-Rabhas) were screened. The non-Rabhas were mainly Tamang, Chetri, Karjee (Nepalis) and Oraon. Peripheral blood samples (3 ml) were collected in EDTA vials from 234 Rabhas [male: 144 (61.54%), age range 1-53 yr; female: 90 (38.46%), age range 4-45 yr]. The samples were transported and diagnosed at the departments of Molecular Biology and Hemato-oncology, Netaji Subhas Chandra Bose Cancer Research Institute, Kolkata during April 2010 to October 2011. The study protocol was approved by the ethics committee of the institute. Naked eye single tube red cell osmotic fragility test (NESTROFT) was performed on spot for all individuals. Their family history was also recorded. ABO blood grouping of the collected blood samples was done, using anti-A, anti-B, and anti-D monoclonal antibodies (Eryscreen Total of Tulip Diagnostics Ltd. Goa, India). Diagnosis of carriers and normal was done by complete blood count using Sysmex-KX-21(Selangor, Malaysia) and haemoglobin analysis with quantitation of HbA, HbA2, HbS and HbF on the Variant Haemoglobin Testing system using the “β-thalassaemia short programme” (Bio-Rad Laboratories, Berkeley, CA, USA). Those who were found normal, were excluded from the molecular study. A total of 299 chromosomes obtained from 101 heterozygotes and 99 homozygotes were selected after preliminary tests, for molecular characterization of HbE mutation.

DNA isolation was done following the standard proteinase K-phenol-chloroform method. Mutation studies in the β-globin gene were carried out by technique of ARMS - PCR (amplification refractory mutation system- polymerase chain reaction). This technique used distinct 3’ specific end primers complementary to either the mutant or the normal allele. The primers used for the detection of HbE [β26(B8)Glu→Lys, GAG>AAG] mutation were as described elsewhere. The PCR products were separated in two per cent agarose gel and visualized after ethidium bromide staining. The results were documented using a Gel Documentation system (Bio-Rad Laboratories, Berkeley, CA, USA). Our study revealed that irrespective of age (range 1-53 yr) and sex all the screened Rabhas have positive Rh value. The ABO blood group consisted of group O (n=90, 38.46%) followed by group B (n=72, 30.77%), group A (n=63, 26.92%) and AB (n=9, 3.85%). Among the screened Rabha population, 12.82 per cent (n=30) were married. NESTROFT was performed for all individuals to find the abnormal osmotic fragility of the red blood cell. Of the 234 individuals, 111 (47.44%) were positive, 27 (11.54%) were doubtful and 96 (41.02%) were negative. The efficiency of NESTROFT in the Rabhas using 0.36 per cent buffered saline solution was 69.23 per cent. The sensitivity, specificity and predictive values of positive and negative tests of
The haematological parameters of the screened Rabhas are shown in the Table. HbE heterozygotes showed 11.92 ± 1.44 per cent HbA2+HbE level while HbE homozygous showed 10.92 ± 1.44 per cent HbA2+HbE level. In cases of heterozygous and homozygous HbE, the median value (range) of HbF were 1.0 (0.4 - 2.1) and 2.9 per cent (1.2 - 4.0%), respectively. The results of ARMS-PCR confirmed the mutation at codon 26 that gives rise to the HbE variant (α₂β₂E). A representative 2.0 per cent agarose gel photograph for the HbE mutation detection is shown in the Figure.

Haemoglobin E (α₂β₂E, i.e., α₂β₂6Glu→Lys) is a variant with a mutation at codon 26 (GAG→AAG) causing a substitution of glutamic acid by lysine in the β-globin chain. Among 234 Rabhas, 101 (43.16%) were found HbE carriers, 99 (42.31%) were homozygous HbE and 522 (36.84 per cent, respectively)

**Table.** Haematological parameters of the screened Rabhas

| Phenotype                  | Normal          | Heterozygous HbE | Homozygous HbE |
|----------------------------|-----------------|------------------|----------------|
| Total no. (%)              | 34 (14.53)      | 101 (43.16)      | 99 (42.31)     |
| Mean MCV ± SD (fl)         | 91.86 ± 5.82    | 77.88 ± 7        | 63.75 ± 4.02   |
| Mean MCH ± SD (pg)         | 29.06 ± 1.70    | 23.96 ± 2.33     | 19.35 ± 1.40   |
| Mean RBC ± SD (10^12/l)    | 4.45 ± 0.52     | 4.99 ± 0.51      | 5.65 ± 0.72    |
| Mean RDW ± SD (fl)         | 47.9 ± 5.44     | 42.42 ± 3.02     | 38.97 ± 1.99   |
| Mean Hb ± SD (g/dl)        | 12.91 ± 1.53    | 11.92 ± 1.38     | 10.92 ± 1.44   |
| Mean HbA₂* ± SD (%)        | 3.08 ± 0.21     | -----            | -----          |
| Mean Hb E + HbA₂** ± SD (%)| -----           | 29.44 ± 1.44     | 86.26 ± 1.54   |
| Median Hb F *** (%) range  | 0.3 (0.1 - 1.4) | 1.0 (0.4 - 2.1)  | 2.9 (1.2 - 4.0) |

*MbA₂ = α₂δ₂*, **HbE = α₂β₂E*, ***HbF = α₂γ₂.

MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; RBC, red blood cell; RDW, red cell distribution width; HbF, foetal haemoglobin.
34 (14.53%) were normal. The HbE variant results from the splice site mutation in exon 1 of the β-globin gene. As a result the production of β-globin mRNA is reduced and appears like a mild β-thal mutation. It was first described by Chernoff et al in 1954 and later by others. The sporadic cases of HbE in India were first identified by Chatterjea et al.

In India, HbE is mostly restricted to the northeastern states with an average frequency of 10.9 per cent, highest 22 per cent in Kolkata (West Bengal) and 50 to 80 per cent in Assam. HbE has been sporadically reported from other Indian States such as Bihar, Odisha, Uttar Pradesh, Rajasthan, Gujarat, Goa, Kerala, Tamil Nadu, Delhi and Chandigarh. High frequencies of HbE variant in 10 populations of Assam (20-60%) and in three populations of West Bengal (12-61%) have been reported.

In our present study, high frequency of HbE variant was observed among Rabhas. This is in accordance with the data of previous investigators who have reported high incidence of HbE among other northeast Indian tribes.

Both HbE heterozygous and homozygous exhibit mild hypochromic, microcytic anaemia which is mostly asymptomatic. Most of the heterozygous HbE have low mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values with or without mild anaemia. In case of HbE homozygous, the red blood cell indices resemble those of β-thalassaemia carriers without any phenotypical changes. The red cell distribution width (RDW) is comparatively greater and it ranges from 38.97 to 42.42 fl suggesting the presence of microcytic anaemia among them.

The Hb level in HbE homozygous among other populations of north Bengal, Tripura, Assam and south Bengal was reported to be 8.95 ± 2.35 g/dl, whereas it was found to be 10.92 ± 1.44 g/dl in heterozygous HbE Rabhas in the present study. Moreover, our unpublished data showed that of the 52 screened non Rabhas, there were very few HbE carriers, but the number was too low to arrive at any valid conclusion. Mass awareness, education and genetic counselling are required to prevent the spread of this mutation among this tribal population of West Bengal.

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References

1. Wasi P, Na-Nakorn S, Suwingdumrong A. Studies of the distribution of haemoglobin E, thalassaemias and glucose-6-phosphate dehydrogenase deficiency in north-eastern Thailand. *Nature* 1967; 214: 501-2.
2. Urade BP. Haemoglobinopathies - HbE and HbS among the Gallong tribe of west Siang district of Arunachal Pradesh, *India. Med Sci* 2014; 7: 83-9.
3. Mandal B, Roy M. The Rabha and their social movement (1925-1950): a case study of North Bengal. *J Humanit Soc* 2013; 10: 5-8.
4. Chandra T, Gupta A. Prevalence of ABO and rhesus blood groups in Northern India. *J Blood Disord Transf* 2012; 3: 132.
5. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
6. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of beta-thalassaemia mutations on the Indian subcontinent: the basis for prenatal diagnosis. *Br J Haematol* 1991; 78: 242-7.
7. Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of beta-thalassaemia: studies in Indian and Cypriot populations in the UK. *Lancet* 1990; 336: 834-7.
8. Bhattacharyya D, Mukhopadhyay A, Chakraborty A, Dasgupta S, Mukhopadhyay S, Pal N, *et al.* Incidence of the Hb E [β26(B8)Glu→Lys, GAG→AAG] variant in Totos, one of the smallest primitive tribes in the world. *Hemoglobin* 2013; 37: 26-36.
9. Sen U, Dasgupta J, Choudhury D, Datta P, Chakrabarti A, Chakraborty SB, *et al.* Crystal structures of HbA2 and HbE and modeling of hemoglobin delta 4: interpretation of the thermal stability and the antisickling effect of HbA2 and identification of the ferrocyanide binding site in Hb. *Biochemistry* 2004; 43: 12477-88.
10. Orkin SH, Kazazian HH Jr, Antonarakis SE, Osthrer H, Goff SC, Sexton JP. Abnormal RNA processing due to the exon mutation of beta E-globin gene. *Nature* 1982; 300: 768-9.
11. Chernoff AI, Minnich V, Chonghareonsuk S. Hemoglobin E, a hereditary abnormality of human hemoglobin. *Science* 1954; 120: 605-6.
12. Itano HA, Bergren WR, Sturgeon P. Identification of a fourth abnormal human hemoglobin. *J Am Chem Soc* 1954; 76: 2278.

13. Chatterjea JB, Saha AK, Ray RN, Ghosh SK. Electrophoretic analysis of hemoglobin in Cooley’s anemia (thalassemia). Evidences of interaction of thalassemia gene with that of an abnormal hemoglobin. *Bull Calcutta School Trop Med* 1956; 4: 103.

14. Chatterjea JB. Haemoglobinopathy in India. In: Jonxis JHP, Delafresnaye JF, editors. *Abnormal haemoglobins*. Oxford: Blackwell Scientific Publications; 1959. p. 322-39.

15. Deka R, Reddy AP, Mukherjee BN, Das BM, Banerjee S, Roy M, *et al.* Haemoglobin E distribution in ten endogamous population groups of Assam, India. *Hum Hered* 1988; 38: 261-6.

16. Patel J, Patel A, Patel J, Kaur A, Patel V. Prevalence of haemoglobinopathies in Gujarat, India: a cross-sectional study. *Internet J Hematol* 2008; 5: 1-9.

17. Patne SC, Shukla J. Hemoglobin E disorders in eastern Uttar Pradesh. *Indian J Pathol Microbiol* 2009; 52: 110-2.

18. Das SK, De M, Bhattacharya DK, Sengupta B, Das N, Talukder G. Interaction of different hemoglobinopathies in Eastern India with a view to establish genotype-phenotype correlation. *Am J Hum Biol* 2000; 12: 454-9.

19. Sengupta B, De M, Dasgupta I, Poddar S. Comparative study of haemoglobinopathies in tribal populations of Arunachal Pradesh and Tripura (North East India). *Int J Hum Genet* 2002; 2: 169-72.

20. Aulakh R, Sohi I, Singh T, Kakkar N. Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. *Indian J Pediatr* 2009; 76: 265-8.

21. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bull World Health Organ* 2001; 79: 704-12.