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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[1,2]. *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[3]. They live in small, isolated groups commonly called as *Rabha Basti*. 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)[4]. Diagnosis of carriers and normal was done by complete blood count using Sysmex-KX-21(Selangor, Malaysia) and haemoglobin analysis with quantitation of HbA, HbA_2, 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[5]. Mutation studies in the β-globin gene were carried out by technique of ARMS - PCR (amplification refractory mutation system- polymerase chain reaction)[6]. This technique used distinct 3' specific end primers complementary to either the mutant or the normal allele[7]. The primers used for the detection of HbE [β26(B8)Glu→Lys, *G*AG>*A*AG] mutation were as described elsewhere[8]. 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)[9].

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

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 HbA_2^* ± SD (%)	3.08 ± 0.21	-----	-----
Mean Hb E + HbA_2^{**} ± 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)

*$HbA_2 = \alpha_2\delta_2$, **$HbE = \alpha_2\beta^E_2$, ***$HbF = \alpha_2\gamma_2$.
MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; RBC, red blood cell; RDW, red cell distribution width; HbF, foetal haemoglobin

NESTROFT were 62.5, 100, 100 and 36.84 per cent, respectively.

The haematological parameters of the *Rabhas* are shown in the Table. HbE heterozygotes showed 11.92 ± 1.38 g/dl haemoglobin concentration with 29.44 ± 1.44 per cent HbA_2+HbE level while HbE homozygous showed 10.92 ± 1.44 g/dl haemoglobin concentration and 86.26 ± 1.54 per cent HbA_2+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 ($\alpha_2\beta^E_2$). A representative 2.0 per cent agarose gel photograph for the HbE mutation detection is shown in the Figure.

Haemoglobin E ($\alpha_2\beta^E_2$ *i.e.*, $\alpha_2\beta_2^{26Glu\rightarrow Lys}$) is a variant with a mutation at codon 26 (*G*AG>*A*AG) 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

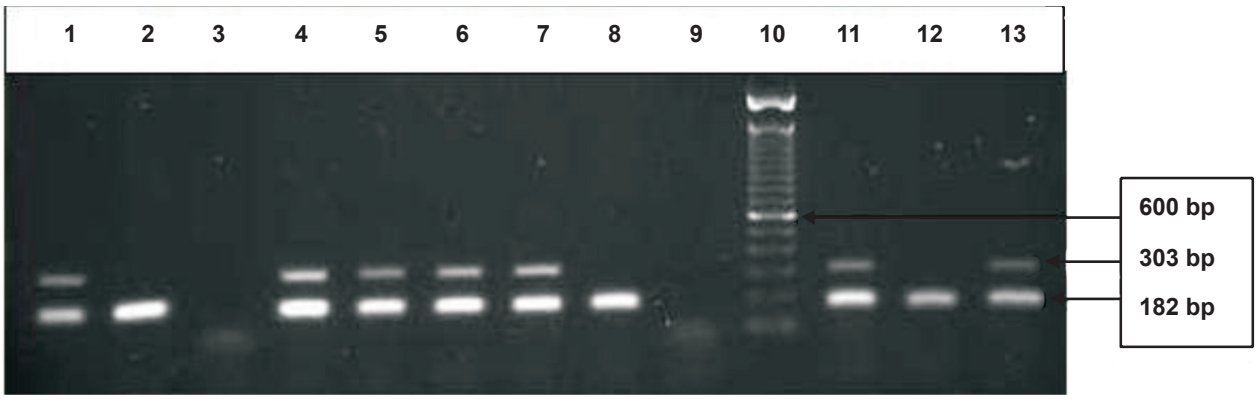

Fig. Representative photograph of the 2.0 per cent agarose gel electrophoresis showing PCR products of *Rabha* samples, analyzed for the presence of the codon 26 mutation. Primers for mutant alleles were used for lanes 1-6 and normal primers were used for lanes 7-9 and 11-13. Lane 1: positive control for the mutant allele; lane 2: negative control; lane 3: non template control; lane 4: HbE heterozygote; lane 5: HbE homozygote; lane 6: HbE heterozygote; lane 7: positive control for normal primer; lane 8: negative control for the normal allele; lane 9: non template control; lane 10: 100 bp DNA ladder; lane 11: HbE heterozygote; lane 12: HbE homozygote; lane 13: HbE heterozygote;. The PCR product size for the internal control band (PAH) and HbE-specific band were 182 and 303 bp, respectively.

34 (14.53%) were normal. The HbE variant results from the splice site mutation in exon 1 of the β-globin gene[10]. As a result the production of β-globin mRNA is reduced and appears like a mild β-thal mutation[11]. It was first described by Chernoff *et al* in 1954[11] and later by others[12]. The sporadic cases of HbE in India were first identified by Chatterjea *et al*[13,14].

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[15]. HbE has been sporadically reported from other Indian States such as Bihar, Odisha, Uttar Pradesh, Rajasthan, Gujarat, Goa, Kerala, Tamil Nadu, Delhi and Chandigarh[16,17]. High frequencies of HbE variant in 10 populations of Assam (20-60%) and in three populations of West Bengal (12-61%) have been reported[15,18].

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[19].

Both HbE heterozygous and homozygous exhibit mild hypochromic, microcytic anaemia[2] 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[20].

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[21], 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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Deboshree M. Bhattacharyya[*], Jayasri Basak[*,†] Soma Mukhopadhyay[*] & Ashis Mukhopadhyay[**]
Departments of [*]Molecular Biology & [**]Hemato-Oncology, Netaji Subhas Chandra Bose Cancer Research Institute (NCRI), 16A Park Lane, Park Street, Kolkata 700 016, West Bengal, India
[†]*For correspondence*: ncri.molecularbiology@gmail.com

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