**INTRODUCTION**

‘Haemoglobinopathies’ are the commonest genetic disorder of predominantly monogenic nature in the world and is a serious public health problem in India. The term ‘Haemoglobinopathies’ is given to the inherited disorders of structure and synthesis of globin part of haemoglobin molecule and falls into several overlapping groups.

Haemoglobin E is the commonest structural variant in South East Asia. It is a haemoglobin variant in which there is a substitution of normal glutamine residue by lysine in the twenty sixth amino acid position of the β-globin polypeptide (β26 Glu to lys) and is caused by the Codon 26 (GAG → AAG) mutation [OMIM NO.: 141900.0071] of the β-globin gene. Except some sporadic appearance in India HbE is confined maximally to persons originating from Eastern India, specially the North-East (Das et al., 2000). HbE mutation among Bodo Kachari population of Assam (North-East India) has been previously reported to be 64.5%, the highest observed frequency of this mutation in the world (Deka et al., 1988).

Tripura is one of the North-Eastern states of India, and is surrounded by the neighbouring country Bangladesh on three sides and connected to the Indian landmass by the Cachar hill region of Assam and the Mizoram state. Population of Tripura in the plains is predominantly Bengali migrants (Austro-Asiatic stock), while the population of Tripura hill region is predominantly tribal population of Tibeto-Burman and Thai stock.

A chance finding of high incidence of Haemoglobin E among tribals of Tripura has already been reported by us (Chakraborty et al., 1996; De et al., 1997). The present study deals with a large cohort of 12 different tribal populations of Tripura and a group of tribal school children for more detailed analyses of the incidence and origin of HbE mutation in these populations using advanced molecular biological methods.

**MATERIALS AND METHODS**

Sample: A total of 840 randomly selected unrelated individuals from 12 different tribal populations of Tripura aged 2 to 76 years were studied during the years 1996-1997 (Table 1) and a group of 196 tribal school childrens (Debarman) aged 12 to 14 years were studied during the years 1999-2000. Inclusion of first order relatives of a selected individual in our study group was eliminated by interview.

A 5 ml blood sample was collected from each individual in vials containing EDTA as the anticoagulant.

Laboratory Analyses: Preliminary screening was carried out by estimating Hb concentration (gm/dl), blood smear examination and red-cell indices with an automated cell counter (Sysmex K-100, Japan). Haemoglobin electrophoresis on agarose gel at pH 8.6 was performed (Chandra et al., 1987) to characterize haemoglobin variants. Fetal haemoglobin was estimated by alkali denaturation method (Dacie and Lewis, 1991, modified after Betke et al., 1959). Slow moving haemoglobin variants like HbA2 and HbE were estimated by the gel elution method (Adhikary et al., 1987).

Homozygosity of Codon 26 (G-A) mutation in Homozygous E individuals was performed by polymerase chain reaction (PCR) based amplification refractory mutation system (ARMS) using the protocol published elsewhere (Chakraborty et al., 1996).

From the cohort of 840 individuals belonging to the tribal population of Tripura, 15 confirmed homozygous E individuals were selected for the study of haplotype of their β-globin gene cluster. The β-globin gene cluster haplotype were determined using four restriction fragment length polymorphism (RFLP) sites, namely, Hind III/Gγ, Hinc II/5′ψβ, Hinc II/3′ψβ for 5′ cluster and Hinf I/3′β (= Bam H I/3′ β) for 3′ cluster. Fragments that contain the polymorphic restriction enzyme digestion using standard procedure (Venkatesan et al., 1992). From the results of RFLP-PCR, four point haplotypes were

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**High Incidence of Haemoglobin-E in Tribal Populations of Tripura, North East India**

Swapan Kumar Das, Madhusnata De, Bani Sengupta, Nikhilesh Das, Dilip Kumar Bhattacharya and Geeta Talukder
constructed using the conventional method (Hundrieser et al., 1988). A total of 30 βE chromosomes were haplotyped.

Statistical Analyses: Suitable statistical methods including mean values and gene frequency was calculated to analyse results.

RESULTS

Screening of 840 individuals from 12 tribal population of Tripura showed 301 individuals to be normal; 398 individuals HbE carriers (heterozygotes), 135 individuals Homozygous E (HbE disease) and six individuals of other categories (including one β-thalassaemia carrier, one HbS carrier and four HbSE compound heterozygote). βE allele frequency in this total study group was 0.4 (Table 1).

Among the 12 tribal population studied, highest βE allele frequency (0.5625) was observed among Mareks (n=64), while the lowest (0.15) was observed among Morasinghs (n=20). In the three populations, namely Marek, Uchai and Darlong, mutant allele frequency (i.e., βE allele) was higher than the normal allele frequency of β-globin gene (Fig. 1).

Mean haemoglobin concentration of Normal individuals was 11.91 gm/dl while in Homozygous E individuals it was 10.82 gm/dl (Table 2).

Analyses of 30 βE or codon 26 (G–A) mutation bearing chromosomes showed that this mutation in tribal population of Tripura was present only on four different haplotype backgrounds all linked to framework 2. Among the four haplotypes, (5’ ++ βE –3’) haplotype was most predominant and was observed on 19 out of 30 βE mutation bearing chromosomes studied (Table 3).

Screening of 196 tribal school children showed 43 individuals to be normal, 113 individuals as HbE carriers and 40 individuals as Homozygous E. βE allele frequency in this study group was 0.4925 (Table 4).
DISCUSSION

Indian populations are subdivided by linguistic, religious, caste and other barriers, which resulted in the existence and perpetuation of thousands of distinct, highly inbred communities. Tripura, the North Eastern state of India has large number of endogamous tribal populations of Tibeto-Burman stock, and have a long history of political and military interaction with different parts of South East Asia. Study of a large sample is thus very useful to interpret genetic heterogeneity among these populations.

840 randomly selected individuals from 12 tribal populations of Tripura is a true representative sample and shows the high incidence of HbE mutation (βE allele frequency = 0.4) in them. It has been seen that individuals homozygous for haemoglobin E may have a thalassaemic phenotype. An impaired synthetic rate, may occur as codon 26 (G → A) mutation could create a cryptic splice site that causes abnormal β-globin mRNA processing and decreased production of mature β-globin polypeptide (Orkin et al., 1982). In our study we have found that homozygous E individuals were not anaemic compared to normal individuals of the same population. This suggests that codon 26 (G → A) mutation is a ‘neutral mutation’ in these populations and also partly explains the predominance of mutant or βE allele in three tribal groups of Tripura.

Heterogeneity of βE allele frequency in different tribal groups can be explained by the endogamy practised by those tribal groups.

RFLP marker based four point haplotyping of 30 chromosomes bearing codon 26 (G → A) mutation was carried out to detect the origin of this mutation. Insipite of expected (2)4 or 16 haplotypes, βE mutations were observed only on four haplotypes, so a linkage disequilibrium exists. (S′+ +βE−3′) haplotype was predominant in Tripura and is also a predominant haplotype in Assam and South East Asia. All the βE haplotypes were linked to framework 2. It is probable that βE mutation in this region originated as a single mutation on (S′+ +βE−3′) haplotype background and appearance of the βE mutation on haplotype backgrounds other than the predominant haplotype may be due to a recombination event in the ‘hot-spot’ region S′ to β-globin gene (Chakravarti et al., 1984).

Our previous study of β thalassemia / HbE matings have shown a distinct preponderance
of HbE in the children in West Bengal (Ajmani et al., 1977). Among the 104 randomly selected individuals of Debarman tribal of mixed age group, $\beta^E$ frequency was 0.4086 ± 0.048, while in 196 school children aged 12 to 14 and belonging to same tribal group, $\beta^E$ frequency was 0.4923 ± 0.036. Apparently this result exhibits an increase in mutant allele frequency, but it does not clearly indicate that selection mechanism is operating in favour of the $\beta^E$ allele and replacing the normal allele of the gene pool by mutant allele. More detailed cross-sectional study of the frequency distributions of HbE in successive age groups involving large number of samples is required to come into any conclusion.

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KEYWORDS: Haemoglobin E, Tripura, Haplotype, PCR-ARMS, RFLP-PCR.

ABSTRACT A large cohort of 840 randomly selected individuals from 12 different tribal groups and a group of 196 tribal school children of Tripura, N.E. India was studied to analyse the incidence and origin of HbE mutation in these populations. $\beta^E$ allele frequency was highest among Marakas (0.5625). In three tribal groups mutant allele frequency was higher than the normal allele frequency of $\beta$-globin gene. Analyses of 30 $\beta^E$ mutation bearing chromosomes shows that this mutation is present only on four different haplotype backgrounds, all linked to framework 2. (5′ ++ $\beta^E$ − 3′) haplotype was most prevalent in the present study group, which indicates the origin of codon 26 (G → A) as a single mutation in this region. Among the 104 randomly selected individuals of Debarman tribals of mixed age group $\beta^E$ frequency was 0.4086, while in 196 school children aged 12 to 14 years and belonging to same tribal group $\beta^E$ frequency was 0.4923. This apparent increase in $\beta^E$ frequency cannot be treated as clear-cut indication of selection of mutant allele.

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