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Distribution of Haemoglobin E among the Bengali Muslim and some Castes of West Bengal

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ABSTRACT

Background and objectives: *Haemoglobin E crystal distorts the red cell membrane to its characteristic shape. Diseases due to this seem to be relatively mild and only in iron deficiency state the haemoglobin E homozygote individuals may be prone to develop severe anaemia. Haemoglobin E provides example of genetic conditions being maintained by balancing selection and polymorphism. This study examines the distribution of Haemoglobin E among the Bengali Muslim and some caste groups namely Paundra Kshatriya, Mahishya and Bagdi of West Bengal.*

Material and methods: *A total of 846 school boys and girls (Paundra Kshatriya: 346, Mahishya: 189, Muslim: 239, Bagdi: 72) whose age is more than 15 years were screened for present study. In this study, we used the Variant, HPLC system with the beta-thalassaemia short program (BTS) to determine the cut-off level for Hb A2 in carriers of classical beta-thalassaemia.*

Results: *The HbE allele frequency of the Muslim, Paundra Kshatriya, Mahisya and Bagdi are 0.031, 0.050, 0.020 and 0.100 respectively. When these results were compared with other populations of the state it depicts that the selective pressure of malaria is not operating among those communities.*

Conclusion: *Considerably high frequency of HbE gene among the Paundra Kshatriya and Muslim populations may be due to their consanguinity of marriage. Though the Paundra Kshatriya, Mahishya and Bagdi all are Hindu by religion they differ with respect of many cultural traits. The studied population however, shows a dissimilar trend with their neighbouring group in respect of HbE gene frequency and do not corroborate the ethnographic background.*

Keywords*: Haemoglobin E frequency, Endogamous Populations, West Bengal*

INTRODUCTION

Discovery of haemoglobin E (HbE) provides example of genetic conditions being maintained by balancing selection and polymorphism. Functionally Haemoglobin E has relatively small oxygen contact area. The severity is marked in case of HbE, where sometime homozygote individuals generally do not survive up to the reproductive age. This abnormal haemoglobin crystal distorts the red cell membrane to its characteristic shape. The haemoglobin E diseases seem to be relatively mild and only in iron deficiency state the haemoglobin E homozygote individuals may be prone to develop severe anaemia. Otherwise, anaemia may be caused by production of target cells (Flatz 1967, Swarup et al. 1965, Cheesbrough and MacArthur 1978, Das et al 1979). The individual with heterozygote HbE has 25 to 40 percent respective abnormal haemoglobin components whereas in homozygous E or S it is 90 to 99 per cent.

Co-existence of three major Haemoglobin variants - HbE, HbS and HbD in India has provided a momentum to the researchers in this country and abroad because they pose a great problem in public health. These genes are considered to have selective advantage against malaria. Many text books on human genetics cite the examples of highest prevalence of Haemoglobin E in Southeast Asia and that for Haemoglobin S in West Central Africa. The incidence of Haemoglobin E gene is 64 percent in Assam and West Bengal populations compared to 10 to 30 percent in some Thailand and Cambodian populations; and that of HbS gene 18 percent in some parts of India compared to 14 percent in some West Central African populations (Mukherjee and Das 1990). The HbE is predominant in North-eastern and Eastern India. Haemoglobin E=HbE ($\alpha_2\beta_2^{\ 26\ Glu\_Lys}$) 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). As such human β-globin gene located on chromosome 11p15.5 is one of the most intensely studied genetic polymorphism of all human loci. It is caused by the Codon 26 (GAG to 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).

The DNA polymorphism present in the haemoglobin cluster is also very interesting and it has also been extensively used to examine human evolutionary history (Das and Talukdar 2001). Haemoglobin E may be present in the heterozygous state (genotype HbAE or

haemoglobin E trait), the homozygous state(HbEE or haemoglobin E disease) and a variety of compound heterozygous states such as haemoglobin E/β thalassemia (HbE/βthal), sickle cell/haemoglobin E disease (HbSE genotype). The beta chain of HbE (β^E) is synthesized at a reduced rate compared with that of normal adult haemoglobin (HbAA=$\alpha_2\beta_2$). This is because the mutation creates an alternate splicing site within an exon. This results in reduced rate of synthesis of β^E chain and therefore of haemoglobin E (HbE), and consequently heterozygotes, compound heterozygotes and homozygotes show some beta thalassemic features. Haemoglobin E (HbE) may therefore be regarded as a β^+ thalassemia haemoglobinopathy. An attempt has been made in this study to examine the distribution of Haemoglobin E among the Bengali Muslim and some castes of West Bengal.

MATERIAL AND METHODS

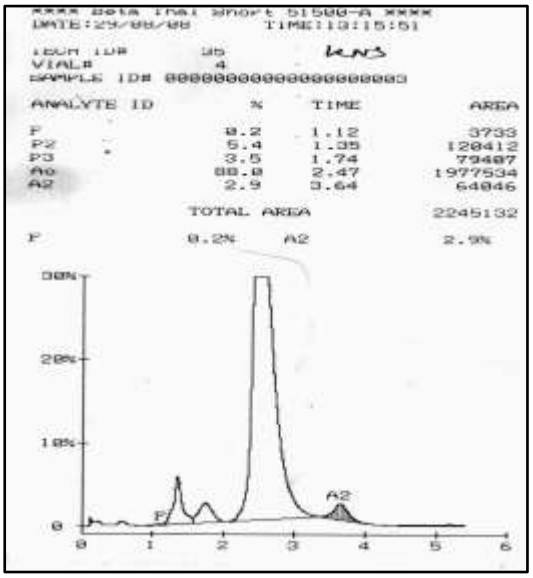
This was a retro prospective study carried out in the Anthropological survey of India, Head Office, Kolkata. A total of 1200 School boys and girls whose age is more than 15 years were screened for present study. A 5 ml of whole blood sample was collected in B.D. vacutainer containing spray dried K_3EDTA anticoagulant. At first samples were screened through Automated Electronic Cell Counter by Beckman Coulter A^cT^8 made in Germany. These included some of the cases of microcytic hypochromic anemia (MCV<80 fl, MCH<27 pg and RBC count >5million/µl) suspected cases of haemoglobinopathy and thalassemia for screening (Wintrobe 1981). Red cell indices were measured on an automated haematology analyzer (Beckman Coulter A^cT^8). In this study, we used the Variant, HPLC system with the beta-thalassaemia short program (BTS) to determine the cut-off level for Hb A_2 in carriers of classical beta- thalassaemia. HbA_0, HbA_2, HbF, and other haemoglobin variants were studiedby HPLC method used for chromatographic separation of human haemoglobin according to their corresponding retention time.

The samples are automatically mixed and diluted on the Variant II Sampling Station (VSS) and injected to the analytical cartridge. The VariantII Chromatographic Station (VCS) dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where HbA_2/F are separated based on their ionic interaction with the cartridge material. The separated HbA_2/F then pass through the flow cell of the filter photometer where the changes in absorbance at 415 nm are measured. An additional filter at 690 nm corrects the background absorbance. The Variant II CDM Software performs reduction of raw data collected from each analysis. To aid in the interpretation of results, windows have been establishedfor the most frequently occurring haemoglobins based on the characteristic retention time. For each sample report and a chromatogram are generated by CDM software showing all haemoglobin fractions

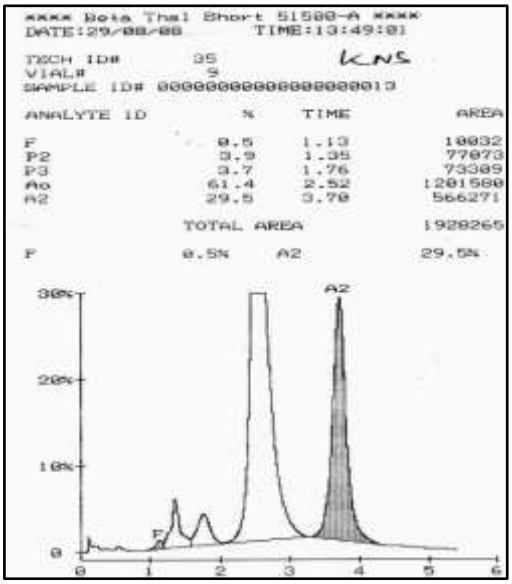
eluted according to their retention times, the area of the peaks, and values of the fractions.

Reports and chromatograms generated were studied and interpreted by observing HbA_2 and F concentration for beta thalassemia and retention time and area percentage of other peaks and windows for structural variants. Each chromatogram shows peaks of HbA_0, HbA_2 and HbF along with C window, D window, S window and two minor peaks, P_2 and P_3. Several haemoglobin variants elute same window; they were provisionally diagnosed by retention time and area percentage keeping in mind the ethnicity of the patient.

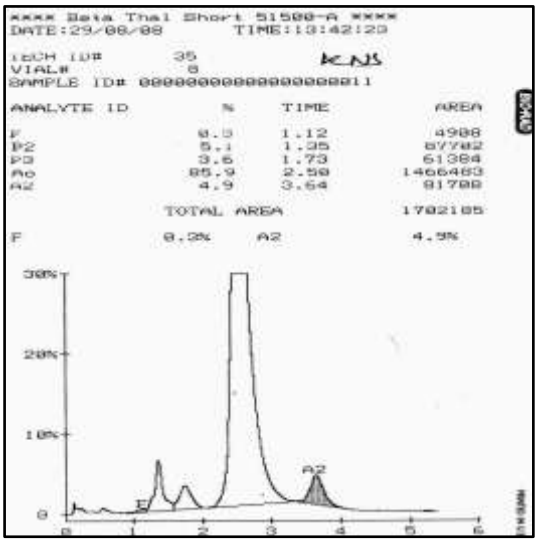
Different features of the chromatograms



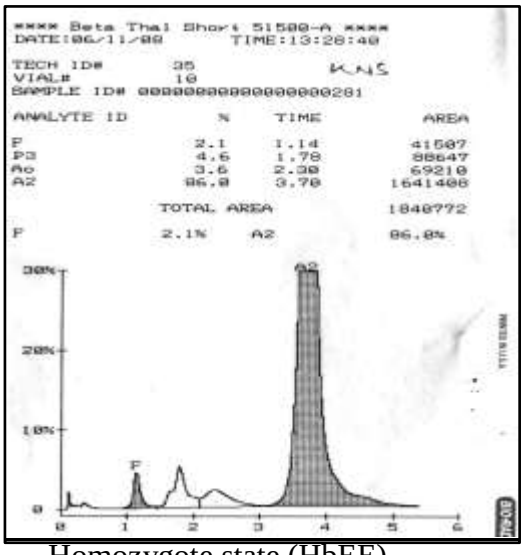
Normal (HbAA)



Haemoglobin E trait (HbAE)



Beta thalassaemia trait (Hb Aβ)



Homozygote state (HbEE)

RESULTS AND DISCUSSION

The table 1 shows the genotype, allele frequencies and Hardy-Weinberg chi-square (HWX^2) values among the populations studied. Among the Paundra Kshatriya 346 numbers of individuals were tested. The genotype frequency of HbAA is 290 and HbAE is 29. The normal allele (A) frequency is 0.950 and Hb E allele frequency is 0.050. The Hardy-Weinberg chi-square value is 0.721. Similarly, in case of the Mahishya 189 individuals were tested. The genotype frequency of HbAA is 164 and HbAE is 7. The normal allele (A) frequency is 0.980 and HbE allele frequency is 0.020. The Hardy-Weinberg chi-square value is 0.070. In case of the Muslim number of individuals tested is 239. The genotype frequency of HbAA is 210 and HbAE is 15. The normal allele (A) frequency is 0.973 and HbE allele frequency is 0.031. The Hardy-Weinberg chi-square value is 0.270. Total individuals tested in case of the Bagdi are 72. The genotype frequency of HbAA is 63 and HbAE is 1. The normal (A) allele frequency is 0.990 and Hb E allele frequency is 0.010. The Hardy-Weinberg chi-square value is 0.218. Among all the populations studied the Hardy-Weinberg chi-square value is not significant, there by indicating that the populations are in equilibrium.

Table 1: Allele frequency, genotype frequency and Hardy-Weinberg chi-square value and p-value in case of Haemoglobin E of the present study

Name of the population groups	Total number(N)	Genotype frequency			Allele Frequency		HWX^2	P-value
		AA	AE	EE	A	E		
Poundra Kshatriya	346	290	29	0	0.950	0.050	0.721	0.396
Mahishya	189	164	7	0	0.980	0.020	0.070	0.079
Muslim	239	210	15	0	0.973	0.031	0.270	0.603
Bagdi	72	63	1	0	0.990	0.010	0.218	1.000

On the basis of allele frequency Nei's genetic distance values are calculated between any two populations and the obtained results is shown in Table 2. It depicts that Muslim and Mahishya are found to be maintaining a close distance (0.623) and Paundra Kshatriya and Bagdi are maintaining a far distance (2.707) in this respect. As this Table is quite self-explanatory it needs no further description.

Table 2: Nei's Genetic D_A (Distance Matrix) among four populations

Populations	Paundra Kshatriya	Mahishya	Muslim	Bagdi
Paundra Kshatriya	0.000	2.030	1.428	2.707
Mahishya	2.030	0.000	0.623	0.677
Muslim	1.428	0.623	0.000	1.292
Bagdi	2.707	0.677	1.292	0.000

Table 3: Distribution of Haemoglobin E among the populations of West Bengal

Sl. No	Population	Total No.	Genotype Frequency			Allele Frequency		HWX^2	p-value	Authors
			HbAA	HbAE	HbEE	A	E			
1	Lepcha (Buddhist)	75	72	3	0	0.980	0.200	0.03	0.863	Saha et al. 1987
2	Lepcha (Christan)	140	134	6	0	0.979	0.021	0.07	0.791	Saha et al. 1987
3	Rava	90	79	11	0	0.939	0.061	0.38	0.538	Kate et al. 1984
4	Rajbanshi	63	50	13	0	0.897	0.103	0.83	0.362	Kate et al.1984
5	Garo	21	19	2	0	0.952	0.047	0.05	0.823	Kate et al. 1984
6	Mech	26	17	9	0	0.827	0.173	1.14	0.286	Kate et al. 1984
7	Toto	116	93	23	0	0.901	0.100	1.4	0.237	Chowdhury et al. 1962
8	Poliya	85	33	52	16	0.694	0.494	0.36	0.549	Das et al. 1990
9	Deshi	103	57	46	40	0.777	0.612	8.51	0.0035	Das et al. 1990
10	Tiyor	95	75	20	2	0.895	0.126	1.13	0.288	Das et al. 1990
11	Oraon	202	202	0	0	0.000	0.000	0	1.000	Saha et al. 1988
12	Brahmin	115	111	4	0	0.983	0.017	0.04	0.842	Ghosh et al. 1981
13	Kayastha	120	117	3	0	0.988	0.013	0.02	0.888	Ghosh et al. 1981
14	Vaisya	71	70	1	0	0.993	0.007	0	1.000	Ghosh et al. 1981

15	Other caste	132	130	2	0	0.992	0.008	0.01	0.920	Ghosh et al. 1981
16	Rarhi Brahmin	42	39	3	0	0.964	0.036	0.06	0.807	Kate et al. 1984
17	Vaidya	29	27	2	0	0.966	0.034	2.06	0.151	Kate et al. 1984
18	Jalia Kaibarta	89	84	5	0	0.972	0.028	0.07	0.791	Kate et al. 1984
19	Lodha	197	178	19	0	0.952	0.048	0.51	0.475	Kate et al. 1984
20	Munda	161	153	8	0	0.975	0.024	0.1	0.920	Kate et al. 1984
21	Bagdi	180	171	9	0	0.975	0.025	0.12		Kate et al. 1984
22	Brahmin	235	235	0	0	0.000	0.000	0	1.000	Choudhuri et al. 1969
23	Kayastha	229	223	6	0	0.987	0.013	0.04	0.775	Choudhuri et al. 1969
24	Vaidya	129	128	1	0	0.996	0.004	0	1.000	Choudhuri et al. 1969
25	Kaora	102	101	1	0	0.995	0.005	0	1.000	Das et al. 1974
26	Podmaraj (Pod)	100	100	0	0	0.000	0.000	0	1.000	Das et al. 1967
27	Santal	102	101	1	0	0.995	0.005	0	1.000	Giri et al. 1982
28	Bhumij	95	95	0	0	0.000	0.000	0	1.000	Giri et al. 1982
29	Poundra Kshatriya	80	74	6	0	0.625	0.037	1.45		Bagchi et al. 2015
30	Poundra	346	290	29	0	0.950	0.050	0.72	0.396	Present Study
31	Mahishya	189	164	7	0	0.980	0.020	0.07	0.079	Present Study
32	Muslim	239	210	15	0	0.973	0.031	0.27	0.603	Present Study
33	Bagdi	72	63	1	0	0.990	0.010	0	1.000	Present Study

Table 3 presents the frequency of HbE gene in the populations of West Bengal. The Vaishya (0.007), Kayastha (0.013) and Brahmin (0.017) show almost no HbE. Among the Deshi (0.612), Poliya (0.494) and Tiyor (0.126) the Hb E allele frequencies reach the levels as high as 0.600. It can be remembered that all these three groups are offshoot of the Bodo group. Side by side, the Rajbanshi, a Koch ethnic group is also characterized with considerably high frequency of this gene (0.1032). The studied population, Paundra Kshatriya (0.0375) show more or less similar E gene incidence (Bagchi et al. 2015) with the Garo (0.0476), Rabha (0.0611) and Christian Lepcha (0.0209) among whom selective pressure of malaria is not operating. It is interesting to note that the Paundra Kshatriya is largest segment of the heterogeneous population in the study area. Other castes residing in Diamond Harbour, South 24 Parganas are the Brahmin, Kayastha, Sadgop, Dom etc. The HbE gene frequency of the Paundra Kshatriya, Mahisya, Muslim and Bagdi are 0.050, 0.02, 0.031 and 0.10 respectively. When these results were compared with other populations of the state it depicts that the selective pressure of malaria is not operating among those communities. Considerably high frequency of HbE gene among the Paundra Kshatriya and Muslim populations may be due to their consanguinity of marriage. Though the Paundra Kshatriya, Mahishya and Bagdi all are Hindu by religion they differ with respect of many cultural traits. The studied populations however, shows a dissimilar trend with their neighbouring group in respect of HbE gene frequency and do not corroborate the ethnographic background.

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