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RELATIONSHIP BETWEEN TOTAL HAEMOGLOBIN — LEVEL AND HAEMOGLOBIN GENOTYPES

ABSTRACT. — In this paper the relationship between total Hb-level and Hb-genotypes has been discussed. The present set of data demonstrates a pattern of a decreasing order of occurrence: A/A > A/E & A/T > E/T > T/T, which suggests no selective advantage for the heterozygotes in a place from where malaria has been eradicated three decades ago.

INTRODUCTION

The total haemoglobin-level varies between sex and between age-groups as well. It is usually higher in males than in females, and it increases with aging (Owen et al., 1973; Das and Mukherjee, 1978; Alekseeva, 1973). But generally it does not further increase significantly with the attainment of adulthood. WHO (1968) suggests certain arbitrary cut-off points for adult males and females, which are 13 gms/dl and 12 gms/dl respectively; and the haemoglobin concentration below the suggested levels may indicate anemia.

The total Hb-level may also be influenced by some environmental factors, such as altitude, nutrition, socio-economic status, etc. (Wolanski, 1973; Ramalingaswamy and Venkatachalam, 1950; Rao et al., 1954 and others). Diurnal fluctuations in the total Hb-level have also been reported (McCarthy et al., 1939; Stengle and Schade, 1957; Mukherjee et al., 1980). The anthropological significance of this trait for ethnogeographical diversities has already been discussed by Garn et al., 1977; Owen et al., 1973; Das and Mukherjee, 1978 and others.

Besides such environmental controls the total Hb-level is also influenced by some genetic factors. Aksoy et al., (1978) and Dincol et al., (1979) have observed through same case studies that the total Hb-level varies from one type of β-thalassaemia intermedia to another. Many authors, on the basis of examination of a few individual cases, have reported that the total Hb-level varies according to the haemoglobin genotypes of individuals. Usually, the total Hb-level is lower in those who have abnormal haemoglobin genes and/or thalassaemia genes than in those with normal haemoglobin genotype, i.e. Hb A/A (Swarup Mitra et al., 1969; Ajmani et al., 1977 and many others).

But no attempt has so far been made to identify the range of variation of the total Hb-level at population level in terms of haemoglobin genotypes. It is known that the heterozygotes with any abnormal haemoglobin gene or with any thalassaemia gene do not normally suffer from anemia. They are much alike the individuals with normal haemoglobin genotypes. But those, who are homozygotes with abnormal haemoglobin genes or with thalassaemia genes, and again others, who are double heterozygotes, do suffer from anemia with varying degree of affliction. In this paper an attempt has been made to examine how and to what extent the total Hb-level varies according to various haemoglobin genotypes compared to normal haemoglobin genotype (i.e. Hb A/A) in the Bengali-speaking population.

MATERIALS AND METHODS

Altogether 206 blood samples, comprising 117 males and 89 females, were collected from the School of Tropical Medicine, Calcutta, during 1980. These were collected in vials dried up with 1 % EDTA. Total Hb estimation was done by Sahli's method within 2—3 hours after the collection of samples.

In determining haemoglobin variants, all the blood samples were run by paper-electrophoresis method, using barbiturate buffer (pH 8.9). Hb F was estimated for each sample by alkali-denaturation technique. All the samples were tested within 24 hours after the preparation of haemolysates. For total haemoglobin estimation, paper-electrophoresis and Hb F estimation, the methods given by Dacie and Lewis (1977) were followed.

Control for this study consists of the data of Das and Mukherjee (1978) for normal Bengali population.

RESULTS AND DISCUSSION

Tables 1 and 2 show the mean total Hb-level with standard error, according to age-groups and genotypes for males and females respectively. The genotypes considered are Hb A/A (normal haemoglobin), β-thalassaemia trait (Hb β-A/T-heterozygote), Hb β-A/E (heterozygote with Hb E variant), Hb β-E/T (double heterozygote), and Hb β-T/T (homozygous β-thalassaemia). It is clearly seen that the total Hb-level increases progressively through the increasing age-groups, i.e. higher the age-group, higher is the total Hb-level. This is, by and large, true for both sex and also for all the genotypes considered.

To have a better understanding of the situation, the entire data have been grouped into two age-classes, i.e. the minor (0—14 years) and the adult (15 + years). The results are set out in *Tables 3 and 4*, for males and females respectively. *Table 3* shows that the mean total Hb-level is considerably higher in the adult age-class in comparison with the minor age-class for all the genotypes. A similar trend is found with the females *(Table 4)*, though it apparently demonstrates that the differences in the total Hb-level between the two age-classes are rather not so high as in males.

Table 5 gives all the t-values indicating difference between various genotypes in relation to the total Hb-level. It is observed that the total Hb-level is significantly higher in the case of those who have normal haemoglobin pattern, i. e. Hb A/A. It is true for both sex — and also for both age-classes. The only exception is that the adult males with heterozygous Hb β-A/E do not differ significantly from the adult males with Hb A/A. It is generally known that the heterozygotes, i.e. Hb β-A/E and Hb β-A/T, behave like normal homozygotes, i.e. Hb A/A, and enjoy somewhat better selective advantage over the normal homozygotes in endemic malarial environments. The results of the present study, considering both sex and both age-class together, do not indeed support the above contention that these two heterozygotes enjoy a better selective advantage (accepting that higher total Hb-level is more selectively advantageous) over the normal homozygotes in a place like Calcutta, from where malaria has reportedly been eradicated three decades ago. And the same trend of no selective advantage has also been available, in respect of the reproductive performance of these two heterozygotes (Bhattacharya et al., 1982), as a supporting evidence.

It is further observed that between the two heterozygotes, i.e. Hb β-A/E and Hb β-A/T, there is no significant difference in the total Hb-level for both age-classes and for both sexes. In general, it is seen that there is significant difference in respect of this trait between any two genotypes carrying gene(s) (either E or T, or both). It is so for both sexes and for both age-classes, excepting for the following four cases: I. between Hb β-A/T and Hb β-T/T in adult males, II. between Hb β-E/T and Hb β-T/T in adult males, III. between Hb β-A/E and Hb β-E/T in minor males, and IV. between Hb β-E/T and Hb β-T/T in minor females. With the present state of knowledge, it is rather difficult to offer any suitable explanation for these observed departures. But plausibly these might be owing to sampling fluctuations.

Finally, it may be noted from the present set of data that the total Hb-level demonstrates a pattern of a decreasing order of occurrence: A/A > A/E & A/T > E/T > T/T, suggesting no selective advantage for the heterozygotes.

TABLE 1. *Total Hb-level (gms/dl) in various age-groups and haemoglobin genotypes (males)*

Age groups	Hb A/A			Hb A/T			Hb T/T			Hb A/E			Hb E/T		
	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.
0—4	8	13.5	0.4	10	9.34	1.22	15	5.76	0.32	3	8.24	1.48	3	6.14	1.06
5—9	13	13.3	0.4	3	9.09	1.78	1	5.22	—	3	9.53	2.13	8	6.90	0.40
10—14	13	14.0	0.3	1	13.20	—	—	—	—	—	—	—	4	7.75	0.55
15—19	15	15.0	0.4	1	14.30	—	—	—	—	—	—	—	3	9.77	1.11
20—24	23	16.2	0.3	3	10.38	2.26	1	9.45	—	—	—	—	1	11.48	—
25—29	11	16.3	0.5	7	12.82	2.64	1	10.50	—	—	—	—	1	10.71	—
30—34	15	15.1	0.4	13	13.52	0.65	—	—	—	1	15.50	—	—	—	—
35—39	11	14.6	0.4	10	13.94	0.42	—	—	—	1	14.20	—	1	9.0	—
40—49	16	15.4	0.3	10	13.51	0.55	—	—	—	4	14.56	0.81	—	—	—
50—59	6	14.8	1.0	1	18.00	—	—	—	—	5	14.61	0.42	1	14.50	—
60—69	8	13.9	0.7	—	—	—	—	—	—	1	16.00	—	—	—	—
70—79	1	12.9	—	—	—	—	—	—	—	—	—	—	—	—	—
80+	2	14.1	0.9	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 2. *Total Hb-level (gms/dl) in various age-groups and haemoglobin genotypes (females)*

Age groups	Hb A/A			Hb A/T			Hb T/T			Hb A/E			Hb E/T		
	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.
0—4	9	13.3	0.1	4	8.43	0.97	5	5.44	0.44	—	—	—	4	6.35	0.48
5—9	7	12.8	0.2	2	9.87	1.52	1	8.70	—	1	8.00	—	3	6.11	0.49
10—14	19	13.0	0.2	1	11.90	—	—	—	—	4	12.73	0.57	3	6.20	0.52
15—19	17	13.8	0.3	1	7.00	—	—	—	—	—	—	—	—	—	—
20—24	21	13.1	0.3	11	11.96	0.58	—	—	—	3	12.85	0.57	1	7.60	—
25—29	7	11.8	0.4	13	11.66	0.46	—	—	—	4	12.56	0.31	—	—	—
30—34	10	12.3	1.0	9	11.94	0.45	—	—	—	2	11.75	0.35	1	6.86	—
35—39	8	12.2	0.7	4	11.25	0.45	—	—	—	4	11.41	1.14	—	—	—
40—49	21	13.7	0.3	5	13.19	1.39	—	—	—	2	12.75	0.44	—	—	—
50—59	7	12.7	0.5	—	—	—	—	—	—	1	12.00	—	—	—	—
60—69	4	13.1	0.5	—	—	—	—	—	—	—	—	—	—	—	—
70—79	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80+	1	10.7	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 3. *Total Hb-level (gms/dl) in two age classes (males)*

Age groups	Hb A/A			Hb A/T			Hb T/T			Hb A/E			Hb E/T		
	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.
Minor (0—14) yrs	34	13.61	0.22	14	9.56	0.99	16	5.73	0.30	6	8.89	1.32	15	6.97	0.36
Adult (15yrs+)	108	15.35	0.24	45	13.41	0.39	2	9.98	0.30	12	14.75	0.40	7	10.70	0.85

TABLE 4. *Total Hb-level (gms/dl) in two age classes (females)*

Age groups	Hb A/A			Hb A/T			Hb T/T			Hb A/E			Hb E/T		
	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.	N	Mean	s.e.
Minor (0—14) yrs	35	13.04	0.11	7	9.34	0.81	6	5.98	0.62	5	11.78	0.96	10	6.23	0.29
Adult (15yrs+)	96	13.05	0.13	43	11.84	0.31	0	—	—	16	12.22	0.20	2	7.23	0.26

TABLE 5. *t values*

Genotypes	Minor		Adult	
	Male	Female	Male	Female
A/A × A/T	5.70*	8.60*	4.31*	4.17*
A/A × T/T	20.74*	19.08*	3.00*	—
A/A × A/E	6.38*	2.80*	0.81	2.44*
A/A × E/T	16.20*	26.19*	4.74*	6.26*
A/T × T/T	3.90*	3.20*	1.84	—
A/T × A/E	0.39	1.94	1.72	0.72
A/T × E/T	2.56*	4.15*	2.60*	3.16*
T/T × A/E	3.43*	5.74*	4.72*	—
T/T × E/T	2.64*	0.48	0.43	—
A/E × E/T	1.92	7.21*	4.94*	7.34*

* *Significant at 5 % level*

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