Human Biology Review (ISSN 2277 4424) 12(1) 2023
Original Scientific Paper (Biswas et al. pp. 59-66)
Revised and Accepted on December 26, 2022

Distribution of beta thalassemia among some endogamous groups of West Bengal

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Citation: Biswas TK, Adak DK, Biswas S, Bharali N, Ray BC and Rao VR. 2023. Distribution of beta thalassemia among some endogamous groups of West Bengal. Human Biology Review, 12 (1), 59-66.

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ABSTRACT

Distribution of beta thalassemia (HbAβ) was studied among the Bengali Muslim and some Bengali caste groups namely Paundra Kshatriya, Mahisya and Bagdi of South 24 Parganas, West Bengal. The allele frequency of the Paundra Kshatriya, Mahisya, Muslim and Bagdi were 0.050, 0.020, 0.031 and 0.100 respectively. These results were compared with published results from other populations of India. The study reveals that selective pressure of malaria is not operating among these populations.

Keywords: Thalassemia, Distribution, Endogamous Populations, West Bengal.

INTRODUCTION

Thalassemia is an inherited blood disorder characterized by less hemoglobin and fewer red blood cells in the body and the carriers are clinically normal healthy individuals. Every year a good number of children are born with β-thalassemia major in different parts of the globe. Most of the affected children are born in the countries where the resources are limited and level of awareness is considerably low. An estimated 7% of the world populations carry a potentially pathological globin gene variant and over 300,000 affected infants are born each year (WHO 1989). Out of these, about 20% are born with thalassemia disease and others include HbAS (sickle cell trait) in Mediterranean and African belt (WHO 1996) and HbAE (haemoglobin E carrier) in mostly Southeast Asian countries including Middle East and Indian subcontinent (Hill 1992, Win et al. 2005).

Large-scale studies undertaken so far do not address the strategy for identifying and counseling carriers for genetic disorders at national or regional level (Colah 1994). Moreover, there has been minimum effort in generating information on the ethnic distribution of the hemoglobinopathies especially in large scattered population with heterogeneous composition. Carrier screening as a part of the prevention curriculum is a prerequisite for any national hemoglobinopathies control program. Therefore, a proper design for sampling of the subjects is necessary, which should be substantiated by a standardized laboratory protocol. Simple field screenings are to be followed up with detailed hematological and other bio chemical assays. Molecular detection is to be utilize wherever required. Looking at the clinical complexity and unavailability of proper treatment-cure facilities and lack of sufficient management, prevention at large scale, remains the only option to arrest the births with hemoglobinopathies in India.

There has been a considerable number of studies reporting the distribution of hemoglobinopathies in India. It is understood that in western and eastern part of the country this disorder is in higher frequency in comparison with rest of the country (Balgir 2000). However, with medical management facilities being made available to the seekers in India a greater number of cases with hemoglobinopathies are continuing longer with minimized clinical severity. Still there has been a constant rise in cases remaining untreated and even undetected. Only population based prospective mode of screening would help in bringing out the exact number of cases to be detected. The problem with many of the current screening and counseling programs is that they do not consider the recipient's socio-psychological background counseling. In majority of cases, it has been seen that after the reporting of cases identified as carrier of any hemoglobinopathies, there has been a notable stigma developing during marriage alliances or decisions of pregnancies. The available alternatives are either too costly e.g., PND (prenatal diagnosis) or misleading (e.g., decision taken by the couples not to go for pregnancies). We understand that the detection and

counseling programs must thereby be preceded with adequate interaction at population level in developing the level of awareness among the participants and target group to be screened as well as the section of the population. Earlier such approaches have been successfully adopted by many countries like Turkey and Iran where there has been an attempt to address this serious health problems by the government and allied organizations (Canatan et al. 2006, Najmabadi et al. 2006). The first of this kind of approach initiated by the Sardinia and Cyprus has been recognized as a landmark. However, the greatest challenge is to introduce these approaches in large communities comprising of different ethnic groups at various level of risk to hemoglobinopathies like India (WHO 1983). This study deals with the distribution of beta thalassemia among some endogamous groups of West Bengal.

MATERIALS AND METHODS

This was a retro-prospective study carried out by Anthropological survey of India, Head Office, Kolkata. A total of 846 school boys and girls (Paundra Kshatriya, Mahisya, Muslim and Bagdi) whose age was more than 15 years were screened for present study. A 5 ml intravenous blood sample was collected in EDTA anticoagulant. At first sample were screened through Automated Electronic Cell Counter 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 hemoglobinopathy and thalassemia for screening. Red cell indices were measured on automated haematology analyzer (Beckman Coulter A^cT^8). HbA_2, HbF, and other haemoglobin variants were studied by HPLC method used for chromatographic separation of human hemoglobin (Kutlar et al. 1984 and Turpeinen et al. 1986). We used the Variant Hemoglobin Testing System (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories Inc., Hercules, CA, USA) under the experimental conditions specified by the manufacturer.

Principle: The Variant II Beta Thalassemia Short Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples were 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 (Clinical Data Management) Software performs reduction of raw data collected from each analysis. To aid in the interpretation of results, windows have been established for the most frequently occurring hemoglobin based on the characteristic retention time. For each sample, report and a chromatogram were

generated by CDM software showing all hemoglobin fractions eluted according to their retention times, the area of the peaks, and values of the fractions.

Sample Collection and Preparation: Five milliliters (5 ml) of whole blood was collected in a vacuum collection tube containing EDTA, which can be stored at 2–8 degrees centigrade for maximum 7 days if processing is delayed. No preparation was required unless the sample was in a tube other than the recommended tube or there was less than 500 μl of sample in the tube. In such case, sample was manually prediluted. Predilution was carried out by mixing 1.0 ml wash/diluents with 5 μl of whole blood sample. HbA_2/F calibrators and normal and abnormal controls were analyzed at the beginning of each run.

Interpretation of Reports*:* 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 Hb A_0, HbA_2, and HbF along with C window, D window, S window,and two minor peaks, P_2 and P_3. Several hemoglobin variants elute same window; they were provisionally diagnosed by retention time and area percentage keeping in mind the ethnicity of the patients.

RESULTS

For the Poundra Khatriya, 346 individuals were tested. The genotype frequency of HbAA was 290 and HbAβ was 26. The normal allele (A) frequency was 0.962 and beta thalassemia allele frequency was 0.037. The Hardy-Weinberg chi-square value was 0.580. Similarly, in case of the Mahisya, the genotype frequency of HbAA was 164 and HbAβ was 18. Normal allele (A) frequency was 0.952 and beta thalassemia allele frequency was 0.047. The Hardy-Weinberg chi-square value was 0.491. Among the Muslim, the genotype frequency of HbAA was 210 and HbAβ was 24. Normal allele (A) frequency was 0.970 and beta thalassemia allele frequency was 0.029. The Hardy-Weinberg chi-square value was 0.230. Whereas, among the Bagdi community, the genotype frequency of HbAA was 63 and HbAβ was 5. Normal allele frequency was 0.965 and beta thalassemia allele frequency was 0.034. The Hardy-Weinberg chi-square value was 0.101 (Table 1). P values of all the chi-squares were not significant at 0.05 level of probabilities.

Table 1: Allele frequency, genotype frequency, chi-square value and p-value in case of beta thalassemia of the present study

Name of community	Total number (N)	Genotype frequency			Allele frequency		Chi-square value	P-value
		HbAA	HbAβ	Hbββ	A	β		
Poundra Kshatriya	346*	290	26	0	0.962	0.037	0.580	0.446
Mahishya	189**	164	18	0	0.952	0.047	0.491	0.484
Muslim	239***	210	14	0	0.970	0.029	0.230	0.632
Bagdi	72****	63	5	0	0.965	0.034	0.101	0.752

*30 individuals were detected as HbE carrier
**7 individuals were detected as HbE carrier
***15 individuals were detected as HbE carrier
****4 individuals were detected as HbE carrier

Distribution of beta thalassemia among the studied population groups of West Bengal was compared with other populations of West Bengal in Table 2. It reveals that the Namasudra with a frequency of 0.075 and Santal with a frequency of 0.0196 show the highest and lowest value in this respect among the population groups of West Bengal. It is interesting to note that out of 2755 individuals tested among the Sadgop, no case of beta thalassemia carrier was reported. Whereas, the Mahisya (0.047), Paundra Kshatriya (0.037), Bagdi (0.034) and Muslim (0.029) of the present study reported a moderate frequency in this respect. The chi-square result showed a statistically significant value in case of the Brahmin (0.046) and Namasudra (0.000).

Table 2: Genotype, chi-Square value and p-value of beta thalassaemia trait among different populations of West Bengal

Sl. No.	Population	District	Total no. of tested	Gene frequency			Allele frequency		Chi-square value	P value	Reference
				HbAA	HbAβ	Hbββ	A	B			
1.	Santal	Midnapore	102	98	4	0	0.980	0.0196	0.04	0.842	Chowdhury et al (1967)
2.	Bhumij	Midnapore	95	87	8	0	0.957	0.040	0.18	0.671	Giri et al (1982)

3.	Brahmin	South24 Parganas	2755	2553	202	0	0.963	0.036	3.99	0.046*	An.S.I. News Letter (2008)
4.	Bagdi	South24 Parganas	2755	2581	174	0	0.968	0.031	2.39	0.122	An.S.I. News Letter (2008)
5.	Kaibratya	South 24 Parganas	2755	2641	110	0	0.980	0.020	1.14	0.285	An.S.I. News Letter (2008)
6.	Kaora	South24 Parganas	2755	2602	153	0	0.972	0.027	2.25	0.134	An.S.I. News Letter (2008)
8.	Namasudra	South24 Parganas	2755	2341	414	0	0.924	0.075	18.18	0.000**	An.S.I. News Letter (2008)
10.	Sadgop	South24 Parganas	2755	2755	0	0	1.00	0.00	0.00	0.000	An.S.I. News Letter (2008)
11	Poundra Kshatriya	South24 Parganas	346	290	26	0	0.962	0.037	0.580	0.446	Present study
12	Mahisya	South24 Parganas	189	164	18	0	0.952	0.047	0.491	0.484	Present study
13	Muslim	South24 Parganas	239	210	14	0	0.970	0.029	0.230	0.632	Present study
14	Bagdi	South24 Parganas	72	63	5	0	0.965	0.034	0.101	0.752	Present study

*Statistically significant
**Statistically significant

DISCUSSION

It is stimulating to note that the Paundra Kshatriya is a major section of the heterogeneous population in the study area. Other castes residing in Diamond Harbour, South 24 Parganas are the Brahmin, Kayastha, Sadgop, Dom, etc. When these results were compared with other populations of the State it depicts that the selective pressure of malaria is not functioning among all those communities. Considerably high frequency of Hbβ gene among the Paundra Kshatriya and Muslim populations may be due to their consanguinity of marriage. Though the Paundra Kshatriya, Mahisya and Bagdi all are Hindu by religion they differ with respect of many cultural traits.

The studied populations (Paundra Kshatriya, Mahisya, Bagdi and Muslim) show a moderate frequency of beta thalassemia, varies between 0.029 and 0.047. These populations however, show a dissimilar trend with their neighbouring groups in respect of Hbβ gene frequency and do not corroborate the ethnographic background (Mohanty et al. 2013; Saha et al. 2021). This gene frequency among the Santal is 0.012 (Chowdhury et al. 1967) and among the Bhumij is 0.040 (Giri et al. 1982).

The prevalence or incidence of occurrence of Beta-thalassemia in India is quite severe than compared to other developed part of the world (Lau et al. 1997; Vogiatzi et al. 2006; Belhoul et al. 2013). Belhoul et al. (2013) found that Iron overload related complications among their patients with thalassemia major were different from those reported internationally. According to Lau et al. (1997) despite the availability of hospital-based screening and prenatal diagnosis for many years in Hong Kong, many women carrying fetuses at risk for thalassemia are not referred for genetic counseling. A community-based program

of education, screening, and counseling is needed in Hong Kong and southern China. Level of awareness regarding beta thalassemia found to be poor among the studied populations. It depicts from the study that selective pressure of malaria is not functioning among all those communities.

ACKNOWLEDGEMENT

This work is based on the project, "Human Genetics Extension Programme", approved by the Anthropological Survey of India, Kolkata. The authors are grateful to the organization. Authors are grateful to all the people of Paundra Kshatriya, Mahishya, Muslim and Bagdi subjects who participated in this study.

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