Prevalence and distribution of Thalassaemias and other Hemoglobinopathies in Nadia District of West Bengalof India: Experience of 1,44,459 cases

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***Abstract*—We evaluated population screening programs (2015–2025), conducted by the Ranaghat Thalassaemia Detection Centre (an Auxiliary unit of STCPWB), Ranaghat, Nadia, West Bengal, India, for the first time in Eastern India in different Blocks of the Nadia district of West Bengal, for prevention of thalassemia comprising screening of heterozygotes and β-thalassemia intermedia (β-TI) cases [β+, β++, β0/β+, βE/βE (codon 26 or HBB: c.79G4A), Hb-E-β-thalassemia (Hb E-β-thal)]. Among 1,44,459 cases, we found 12,002 heterozygotes and 50β-TI individuals (who had no information about their disorders). Results were evaluated with standard haematological analyses including erythrocyte indices, haemoglobin (Hb) typing and quantification. Participants were divided into three groups (high school students, college students andantenatal women). The objectives of this evaluation were to fix cut-off values of red blood cells (RBCs), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), red blood cell distribution width (RDW) and Hb A2, as the standard World Health Organization (WHO) guidelines were not strictly followed in mass-scale screening programs. We have observed many dilemmas in considering the status of the thalassemia subject, due to presence of some other clinical conditions such as iron deficiency anaemia, β-thalassemia (β-thal), β-thalassemia (β-thal), clinically silent Hb variants, along with thalassemia. The MCV values varied greatly in different conditions of hemoglobinopathies, whereas MCH provided a more stable measurement. We found an MCH value of<27.0 pg is a suitable cut-off point for screening in this population. Participants with an MCH of<27.0 pg should be investigated further to confirm or exclude a diagnosis of β-thal trait/Hb variant.33**

In rural areas of West Bengal, the most common Hb variants detected were Hb E traits and then β-thal traits. In view of the high prevalence of HbE hemoglobinopathies in this region, routine premarital screening and genetic counselling should be emphasized and encouraged to prevent the birth of a thalassaemic child (combination of beta with HbE), and thus curtailing the burden on families and the health economy.

***Index Terms*—β-Thalassemia (β-thal), Hb A2/E, Hb E-β-thalassemia (Hb E-β-thal), Mean corpuscular Hb (MCH), Mean corpuscular volume (MCV), Population screening.**

I. INTRODUCTION

India is a country comprising 1.44 billion people, 17.78% of the global population **(1)** and the largest populated country of the world and has a huge diversity in its population structure due to difference in ethnic, religion, language and geography **(2).** Most of the communities of India prefer to marry within their community; the noncommunicable diseases (genetic diseases) show great prevalence in some of the communities, while these noncommunicable diseases are either absolutely absent or present in different forms in other communities.

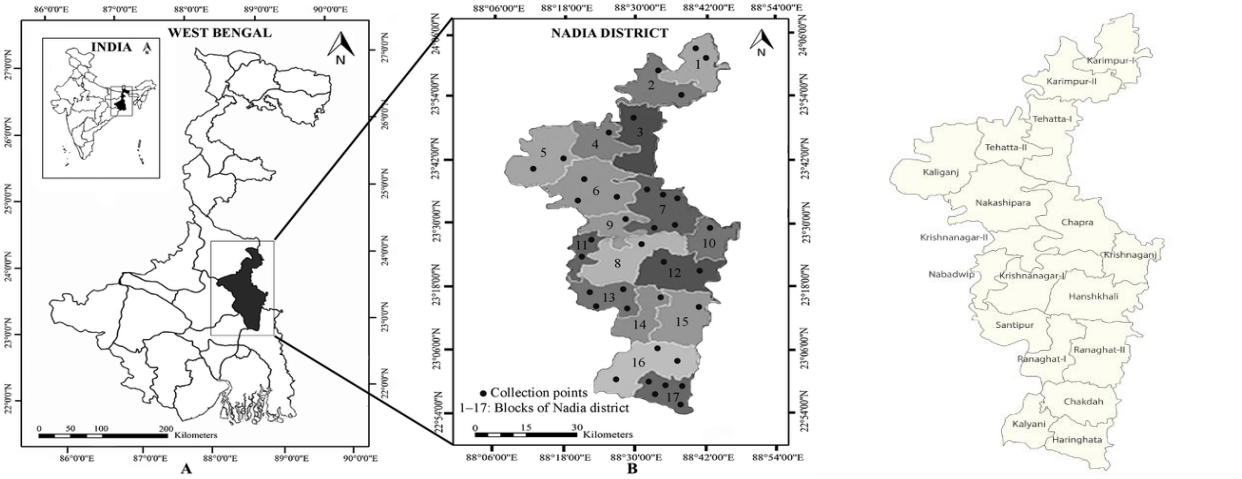
The current national infant mortality rate in India is 28 per 1000 births (1). The contribution of communicable diseases in the infant mortality rate is decreasing day by day due to implementation of a nationwide program (National Rural Health Mission) ensuring better health care facilities for neonates. Better primary neonatal health care has ensured fewer infant deaths due to communicable diseases. Thus, there is an increasingly rapid transition in the burden of disease across all age groups from primarily communicable to noncommunicable diseases, which has been reflected by an increasing percentage of deaths due to noncommunicable diseases. According to the study report "India: Health of the Nation's States"- The India State-Level Disease Burden Initiative in 2017 by Indian Council of Medical Research (ICMR), it is estimated that the proportion of deaths due to Non-Communicable Diseases (NCDs) in India have increased from 37.9% in 1990 to 61.8% in 2016 (3).In 2019, the World Health Assembly extended the WHO Global action plan for the prevention and control of NCDs 2013–2020 to 2030 (4).Thalassemia and hemoglobinopathies are the most common inherited noncommunicable disorders in humans and they represent one of the major public health problems in many parts of the world including India (5).It has been estimated that the prevalence of pathological hemoglobinopathies in India is 1.2 per 1000 live births (6), and births per year is being estimated at approximately 27.72 million (1). These figures would suggest the annual birth of 33,264 babies with a serious haemoglobin (Hb) disorder. In 1989, the World Health Organization (WHO) Working Group had released guidelines for the control of Hb disorders with an estimated value of 3.9% carrier frequency for b-thalassemia (β-thal) in India, encompassing all types of β-thal trait (7). A WHO update on β-thal in India indicated a similar overall carrier frequency of 3.0–4.0%, which, given the current national population, would translate to between 35.1 and 46.8 million carriers of the disorder nationwide (8,9).

Screening projects have been initiated in different states of India with a sizable number of thalassemia carriers and patients. Reports have been communicated from different population screening data from different parts of India comprising West, North, East and South (10). These different studies show different carrier frequency estimates and unfortunately, there are no adequately representative data sets to confirm or deny these average disease prevalence estimates, which could realistically be applied to each and every community and sub-population. Overall, the carrier frequency for β-thal ranges from 0.3 to 17.0% in different local communities (5,8,11,12). There are basically too few data presented from West Bengal (13–15) and population samples were too small to comment on the thalassemia carrier frequency in about 9.1 million people living in West Bengal (16).

Among other structural variants of Hb, HbE (βE or HBB: c.79G4A) is one of the common, along with different countries of Northeast Asia including Thailand, Laos and Cambodia (17,18). Here, the prevalence of β-thal is also fairly common. Thus, the βE allele often combines with other mutated β-thal alleles to produce the compound heterozygous condition of HbE-β-thalassemia (HbE-β-thal) (19). This HbE-β-thal is the most common type of thalassaemia in West Bengal along with Bangladesh, the adjacent country to this state and is involved in a huge amount of migration. HbEβ-thal shows a great variety in terms of clinical manifestation of the disease leading from asymptomatic, moderate to severe transfusion-dependent anaemia.

To throw some light on the thalassemia carrier frequency and also to have an estimate on the disease burden in this state, we evaluated population screening programs (2015 – 2025), conducted by the Ranaghat Thalassaemia Detection Centre,run by National Health Care charitable Trust (an Auxiliary unit of STCPWB),situated at T. B. Hospital, Srinathpur Ranaghat, Nadia, West Bengal in the eastern part of India particularly in this Nadia district of West Bengal(Figure 1), as this district is adjacent to Bangladesh and so many migration occurred here, especially in this district. The motto was the prevention of thalassaemia comprising screening of heterozygotes and β-thal intermedia (β-TI) cases (β+, β++, β0 /β+, βE /βE, HbE-β-thal), the objective being to reduce and eventually prevent thalassaemia in Eastern India by promoting thalassaemia carrier screening programs in the general population and affected families.

Figure 1. Location map of Population screening area in Nadia District, West Bengal, India



II. MATERIALS AND METHODS

Participants were children of higher secondary schools and colleges who divided into three groups (group I: school children of standard 9, 10, 11 and 12, group II: college students, group III: antenatal cases). These divisions were done to maximize the chances of reaching the roots of the disease. The most emphasis was given on group II, because if the young boys and girls get the information about their thalassaemia status before marriage, they can decide accordingly and eventually prevent the birth of β-thal major (β-TM) children. Emphasis was also given to group III (pregnant women) because if the woman knows the thalassemia carrier status of her husband and herself, and if both are found to be carriers, then, she could opt for prenatal diagnosis (PND) to find out the thalassaemia status of the fetus. If the fetus is found to carry β-TM, then she could always opt for medical termination of pregnancy, providing it is within the stipulated period of gestation according to the existing act of Medical Council of India. Initially, the level of awareness in people of West Bengal was not great, so that there were not enough participants in the screening programs. But now state governments of India are taking initiatives and passed compulsory testing of blood for Haemoglobinopathies as the frequency of traits (both beta and HbE along with some other Hb variants) are increasing day-by-day.

Sometimes we also found that thalassaemia disease or even carrier status is a social stigma to the people. As our goal is to address the maximum number of carriers in the screening programs, and to prevent the further births of thalassaemic children, group II (college students) were an easy target population to reach the maximum number of carriers likely to give birth to thalassaemic children, until or unless it can be ensured that they marry normal partners. For a state like West Bengal, where there is great diversity of cultures, heritage, religion and socioeconomic structures, it is very difficult to address people from every class of the society by a single strategy. That is why different strategies were implemented in different situations to maximize the chance of addressing the affected and carriers.

Written consent for evaluation of thalassaemia and other haemoglobinopathy status was obtained from adult participants as per guidelines of the institutional ethics committee. Peripheral blood samples were collected from every participant in the various screening camps, in vials containing 5 mM EDTA. The handling of all human blood samples was carried out in accordance with the guidelines established by the Local Ethics Committee. Basically three levels were employed for screening the population. The initial screening was done by Hb and complete blood count (CBC) in an Automated Haematology Analyser (Cell Counter: Acculab CBC 360 neo 530; Acculab Biomedical Pvt Ltd, Mumbai, India and ABX Micros ES 60 : Horiba Medical, Montpellier Cedex 4, France) using the manufacturer's protocol. The participants were evaluated for Hb, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red blood cell distribution width (RDW) and haematocrit or

packed cell volume (PCV). The complete and final screening was done through Hb variant analysis by high performance liquid chromatography (HPLC). Haemoglobin variants (Hb A, Hb F and Hb A2/E) were estimated by HPLC (VARIANT II; Bio-Rad Laboratories, Hercules, CA, USA) using the manufacturer's protocol. The screening was done following standard WHO guidelines for interpreting HPLC and CBC data.

III. RESULTS

One lakh forty-four thousandfour hundred fifty-nine people participated in different screening camps conducted by the Ranaghat Thalassaemia Detection Centre (an Auxiliary unit of STCPWB), Ranaghat, Nadia, West Bengal, were screened for their thalassaemia and other haemoglobinopathy status; the results of these screening programs for Nadia district of West Bengal are summarized in **Table 1.**

Table 1. Screening results year-wise in Nadia district of West Bengal, Eastern India.

Year	n	Patient	Normal		Carrier%
2025	4197	02	3813	382	9.1%
2024	8969	15	7777	1177	13.1%
2021	21401	15	19380	2006	9.3%
2020	12039	14	11462	1274	10.6%
2019	24001	00	22237	1764	7.3%
2018	19306	00	17830	1476	7.6%
2017	20601	01	18991	1609	7.8%
2016	23118	00	21494	1624	7.0%
2015	10827	03	9423	690	6.4%
Total	144459	50	132407	12002	8.30%

Overall carrier frequency from the cumulative data of this Nadia district, showed that the carrier frequency was 8.30% (Figure 2). The frequency of asymptomatic or less symptomatic patients (β-TI, β-thal minor, and HbE cases) (β+, β++, β0 /β+, βE /βE, HbE--thal) was found to be quite low (0.03%) (Figure 2) as all the participants were asymptomatic ones. The carrier frequency in this district shows considerably lower (Table 1), whereas the other districts of West Bengal show higher(20). Sex distribution among the participants (normal, patients and carriers) is summarized in **Table 2.**

Figure 2. Overall distribution of normal, patients and carriers in the population (n = 144459).

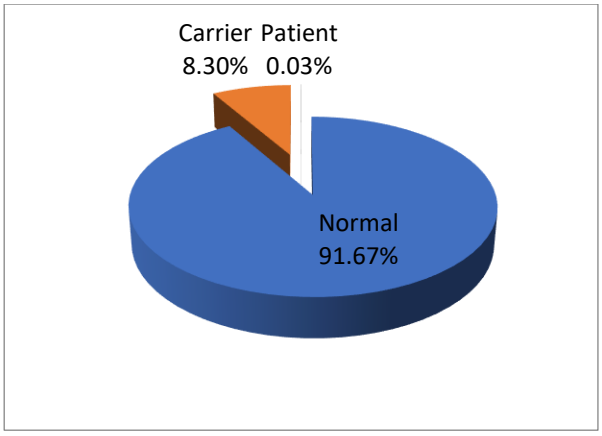


Table 2. Sex distribution of patients and heterozygotes/carriers observed in the population of Nadia district of West Bengal, Eastern India (n = 144459).

Year	Patient		Carrier	
	Males	Females	Males	Females
2025	00	02	138	244
2024	05	10	318	858 (Trans=01)
2021	09	06	835	1171
2020	05	09	412	862
2019	00	00	839	925
2018	00	00	728	748
2017	01	00	669	940
2016	00	00	938	686
2015	02	01	383	307
Total	22	28	5260	6741
	(44%)	(56%)	(43.8%)	(56.2%)

Overall, 61,073 males and 71,334 females participated in the study, making the male:female ratio 1:1.2. This ratio is more or less same for normal and carriers (1:1.3)and patients (1:1.3).These data can be interpreted in a very interesting way. Initially, when the screening programs were set up, few female participants came for evaluation. But as time goes by and awareness becomes more widespread, the female participation ratio increases considerably. Finally, female participants become morethan the male participants, which help to prevent birth of Thalassaemic child in this district as there are so

many teen-age married girls (Table 3) as well as teen-age mothers (Table 4)participated in this screening program.

Table 3. Screening results of Married Girl cases in Nadia district of West Bengal (n=3831)

Normal	<18 yrs	Carrier	<18 yrs	Patient	<18 yrs
3408 (88.95%)	57 (1.48%)	417 (10.88%)	6 (0.15%)	6 (0.15%)	1 (0.02%)

Table 4. Screening results of Antenatal mother cases in Nadia district of West Bengal (n=15936) [Normal = 14211]

Total No	HbA0	HbA2	HbF	HbE	HbD	HbS	Hb	MCV	MCH	RDW
Carrier 1718 (10.78%)	74.65± 1.94	6.30± 3.67	1.11± 0.19	20.98± 12.15	15.3± 21.67	36.07 ± 1.31	10.25± 0.30	78.85± 3.28	25.50± 1.11	13.05± 1.80
Patient 07 (0.04%)	58.39± 47.38	63.2± 52.28	2.63± 2.91	-	-	8.96± 1.30	68.9± 16.55	25.13± 5.30	12.46± 2.23	63.2± 52.28

The most important focus of any screening program are the heterozygotes or carriers and the asymptomatic or less symptomatic β-TI, β-thal minor and HbE patients. Thus, we studied these data in more detail; HbE were found to be the most frequent allele in this district, comprising about 53.2% of heterozygotes in Nadia, as this district is adjacent to Bangladesh, where HbE haemoglobinopathy prevails. The distribution of different disease alleles [HbE, β-thal, HbD-Punjab (HBB: c.364 G4C) HbS (HBB: c.20A4T)] and other variants in this district of West Bengal is given in Table 5. In 12002 heterozygous/carriers, β-thal trait was found in 33.25% cases and HbE trait was seen in 53.22% cases (Figure 3). HbS and HbD-Punjab were found in only about 3.5% of the heterozygotes. 204 (1.7%) cases carried sickle cell trait and 219 (1.8%) cases carried HbD-Punjab trait in 12002 heterozygotes (Figure 3). As HbE (53.2%) and the β-thal (33.25%) alleles comprise about 86.5% heterozygotes, so the prevalence of HbEE and HbE-β-thal should be higher in this Nadia district of West Bengal population. Therefore, patients of this district are mostly of Intermedia type, who are less transfusion-dependent.

Figure 3. Distribution of disease alleles (b-Thal, Hb E, Hb D-Punjab and Hb S) in heterozygous carriers (n =12002).

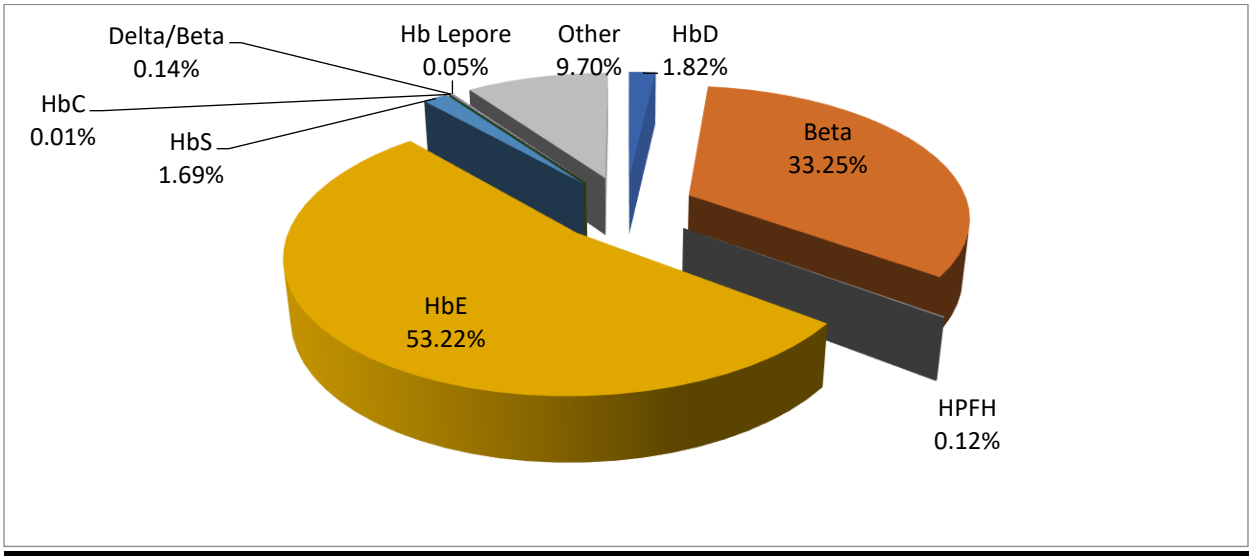


Table 5. Distribution of different haemoglobin variant traits/carriers observed in the population of Nadia district of West Bengal (n =12002).

Year	HbD	Beta	HPFH	HbE	HbS	HbC	Delta / Beta	Hb Lepore	Other
2025	2	99	2	204	5	0	4	0	66
2024	19	228	1	393	21	0	8	0	507
2021	12	687	8	962	16	0	3	4	314
2020	22	365	4	567	33	0	3	3	277
2019	31	646	0	1054	33	0	0	0	0
2018	35	541	0	872	28	0	0	0	0
2017	52	563	0	964	29	1	0	0	0
2016	32	600	0	966	26	0	0	0	0
2015	14	260	0	403	13	0	0	0	0
Total	219 (1.8%)	3989 (33.2%)	15 (0.1%)	6385 (53.2%)	204 (1.7%)	1 (0.01%)	18 (0.14%)	7 (0.05%)	1164 (9.7%)

IV. DISCUSSION

Results were analyzed to establish which of the variables (MCV and MCH) were more suitable for screening and to determine suitable cut-off points for measuring the Hb A2/E percentage. The MCH was superior to the MCV (as it varies greatly in different haemoglobinopathy conditions) for thalassaemia screening as it was a more stable measurement. As in this population, the HbE traits and HbEE patients are very common, so here the MCV values cannot help to exclude traits/carriers even in case of some patients, since in so many cases the MCV values are more than normal **(Table 6).**An MCH level of <27.0 pg can be taken as a suitable cut-off point for screening**(Table 7).**Pregnant women presenting at any antenatal clinic with an MCH level of <27.0 pg should be investigated further to confirm or exclude a diagnosis of thalassaemia trait. The investigative recommendations, which were followed strongly includes the following: all pregnant women were tested for β-thal trait and also for other Hb variants (HbE and HbS for the Eastern Indian population), even though the MCV and MCH levels were more than 75.0 fL and more than 27.0 pg. When the MCH value was <25.0 pg, selected pregnant women were tested for β-thalassemia (β-thal), especially when the person concerned appeared to be ‘‘normal’’ after being screened for β-thal trait and HbE or HbS. Again, in our opinion, the RDW Index is also one of the best index as it provides a sensitivity and specificity between 90.0–100.0% to distinguish iron deficiency anaemia from thalassaemia.

Table 6. Distribution of different blood parameters in HbE variant traits/carriers and patients observed in the population of Nadia district of West Bengal (n =6427).

Type	Total no.	HbA0	HbA2/E	HbF	Hb	MCV	MCH	RDW
HbE/+	6385	69.23± 24.75	27.04± 2.67	0.71± 0.73	11.88± 2.34	80.98± 16.63	26.18± 6.03	13.90± 2.34
HbEE	42	8.794± 5.73	79.974± 8.54	5.878± 2.59	10.13± 1.95	67.496± 4.22	21.6± 3.56	16.352± 3.63

Table 7. MCH values of different haemoglobin variant traits/carriers observed in the population of Nadia district of West Bengal (n =12002).

Type	Total Nos.	MCH
Beta	3989	21± 2.84
HbE	6385	26.18+6.03
HPFH	15	24± 1.57
HbS	204	25± 2.73
Delta/Beta	18	23± 4.57
Hb Lepore	7	22± 0.49
HbD	219	27.41± 2.72
HbC	1	30
Other	1164	29± 2.32

V. CONCLUSIONS

It is essential to select precise methodologies to detect thalassaemia and other haemoglobinopathies in our population here. Genetic counseling should be according to the age of the individual and the target groups need to be screened. Genetic counselling should be based on genotype or mutation of parents to avoid PND. Overall, it is critical to plan an adequate strategy and to choose a single suitable laboratory method for carrier identification in this pocket of our country, where resources are limited. As local government has fixed guideline to test the individuals at free-of-cost by using HPLC method only in those cases where cut-off values of MCV is less than 85. Some unknown Hb variants have also been reported **(Table 4),** which can affect the surety of success in PND. Frequency of all these factors may vary considerably in different districts. There is a need to establish a systematic screening program, which should be proposed at different times in life that would help investigators to avoid misdiagnosis and to improve screening for heterozygotes and some β-TI cases in a cost-effective way.

Declaration of interest

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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