Community Genetics and Health: Haemoglobinopathies in India

National project undertaken during 2005-2012

ANTHROPOLOGICAL SURVEY OF INDIA, MINISTRY OF CULTURE, GOVERNMENT OF INDIA

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Foreword

Anthropological Survey of India was recognized as a dedicated organization for advanced research in the field of anthropology in India. The research activities of the Survey are oriented towards both basic science as well as to applied aspects, which have contemporary relevance and of national significance.

Some of the major diseases of genetic orientation reported to be occurring in the Indian population are sickle cell anaemia, alpha and β-thalassemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency. Studies on genetic disorders are gaining prominence as they have profound health implications for various communities in India. However, the large-scale studies undertaken so far do not address issues relating to counselling carriers for genetic disorders. Moreover, there has been minimum effort in generating information on the ethnic distribution of the haemoglobinopathies especially in large, scattered population with heterogeneous composition. Carrier screening as a part of the prevention curriculum was a prerequisite for any national haemoglobinopathies control program. The need for a proper design of sampling substantiated with a standardized laboratory protocol was understood. Simple field screenings were followed up with detailed haematological and other biochemical assays. Molecular detection was exerted wherever required. Looking at the clinical complexity and unavailability of proper detection facilities, prevention of disease gene at large scale, remained the only option to arrest the births with haemoglobinopathies in India.

There was a paucity of comprehensive community-wise health research on regional basis in India. Most of the studies are isolated and fragmentary in nature. An urgent need for initiating the area specific, tribe specific, action-oriented health research gave rise to the present study on haemoglobinopathy in India.

In 2005 the project entitled “Community Genetics; Health and Bio-Cultural Diversity”, was initiated by the Survey. A team of trained research scientists participated in this project. Later, the study was continued under the project ‘Community genetics extension programme’. This programme was continued in various geographical locations in the country. The main objective of this project was to find out the prevalence for sickle cell anaemia and other haemoglobinopathies among different ethnic communities of India. Carrier screening was also carried out as a part of the prevention curriculum.

The present report deals with studies on genetic disorders (sickle cell anaemia/thalassaemia) among different ethnic communities of Andaman Islands, Central India, Northeast India and Eastern India. Some of the findings of these research studies in different parts of India are already published in refereed journals by staff of AnSI. Similarly, reports are submitted to the regional centres for the studies conducted in their respective regions. However, it was felt that a consolidated report containing the findings of the studies undertaken by all the regional centres of the survey was desirable. Hence an attempt was made now for such a consolidated report.

Acknowledgment

We sincerely thank Prof. V.R. Rao former Director-In-Charge of Anthropological Survey of India, Ministry of Culture, Dept. of Culture, Government of India for sanctioning and launching this project in 2005.

We express our sincere gratitude to Director, Anthropological Survey of India for his continuous support in the completion of the report and bring it to the present form.

We acknowledge our heartfelt and humble thanks to all the former directors, Prof V. K. Srivastava, Prof. K.K. Basa, Prof. K.K. Misra, Shri G.S. Rautela, Dr. Gauri Basu, Smt. Sanjukta Mudgal, Joint Secretary who had encouraged and helped to carry out the screening and research at the molecular level during the long phase of the project.

We gratefully acknowledge the immense help and cooperation received from the district administration, Chief District Medical Officers, Medical Officers and Staff Nurses and local panchayat members of all the states and district Head Quarters from where the screening was carried out.

We acknowledge the co-operation extended by all the administrative staff of Anthropological Survey of India. We acknowledge all who are directly or indirectly associated with this endeavour, the participants, who relentlessly cooperated and came forward and donated their precious blood samples to make this project a grand success.

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Chapter 01

INTRODUCTION

Community genetics refers to the benefit rendered to the individuals of a community by applying the knowledge of genetics so that the gene pool or the health of the individual and that of the future generation was improved. Inherited genetic disorders in families contribute to the pathogenesis of diseases. The Anthropological Survey of India initiated a large-scale multi-phased mass screening program. The area, which has been given initial focus was the Central India, North-Eastern India, Eastern India and the Andaman Islands.

The present report focuses on the condition which has a direct link between genes and health, that explores to understand the roles of genes responsible for an inherited disorder of the haemoglobin in man.

The structure of human haemoglobin

The haemoglobin, (haem+globin) develops in the cells produced by the bone marrow and forms red blood cells (RBC). Haemoglobin was a protein that transports oxygen from the lungs to the other body tissues and takes away carbon dioxide from the tissues to the lungs. The haemoglobin molecule comprises of four sub-units, each having one polypeptide chain and one heme group. The heme group was surrounded by a globin group, forming a tetrahedral structure (Figure 1). The heme contains iron and the globin which are chains of polypeptides contains specific amino acids and determine the chemical properties and function of the haemoglobin.

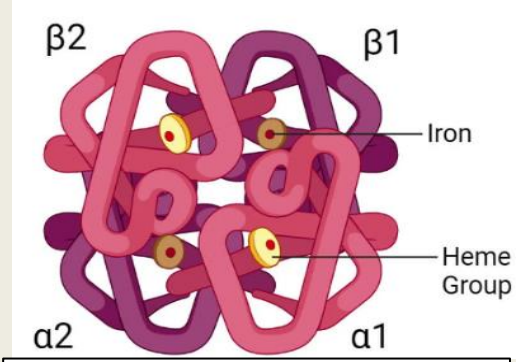

Figure 1.1: Structure of Haemoglobin
(source:https://microbenotes.com/haemoglobin/

The polypeptide chains in adult human are of two types: the alpha chain, encoded by genes on chromosome 16 (containing 141 amino acid) and the β chain containing 146 amino acid residues), and the non-alpha chains, encoded by β genes on chromosome 11. Various polypeptide chains are formed due to the combination of the amino acids, and are designated by the Greek letters, alpha (α), β (β), gamma (γ), delta (**δ**), epsilon (Ɛ), and zeta (ζ).

In early embryonic life, epsilon, zeta, and some alpha chains are synthesized, in foetal life alpha and gamma chains are synthesized, and in postnatal life, alpha, β, and delta chains are synthesized. The three main types of haemoglobin are:

- Foetal haemoglobin (HbF): which include two alpha chains and two gamma chains ($\alpha_2\gamma_2$)

- Adult haemoglobin A (HbA): which includes two alpha chains and two β chains ($\alpha_2\beta_2$).
- Haemoglobin A_2 (HbA_2): which include two alpha chains and two delta chains ($\alpha_2\delta_2$).

HbF was the haemoglobin of foetuses and newborns, the β subunits are replaced by gamma subunits (γ_1 and γ_2). The transition from foetal HbF to HbA begins before birth and is completed by approximately six months of age. The switch from gamma to β chains was regulated by the body carefully to keep the amount of circulating haemoglobin constant. In rare cases the delta subunits (δ_1 and δ_2), are replaced by the β subunits. In late foetal life, HbF, forms about 80–90 percent of the total haemoglobin at birth, whereas in postnatal (postpartum) life, only traces are observed up to 6–12 months, and foetal haemoglobin is replaced by the human adult haemoglobin HbA comprising about 96.5 percent and HbA_2 and about 3.5 percent of the total adult haemoglobin.

The coding for the globin chains of the tetrameric haemoglobin protein is responsible for different variants, that result in altered synthesis of α or β-globin or both and are responsible for the structural changes of haemoglobin. This inherited structural and functional abnormalities in molecular chains of human haemoglobin, are commonly classified as haemoglobinopathies and includes all haemoglobin disorders.

Types of haemoglobin variants

The haemoglobinopathies are the commonest human single gene disorders with an estimated frequency of 7 percent globally carrying the abnormal genetic variant and over 300,000 affected new-born (WHO, 2006).

The haemoglobinopathies may be classified into two broad groups as given in figure 1.2. The structural change in the haemoglobin causes diseases such as sickle cell diseases, haemolytic anaemia, erythrocytosis or polycythaemia (Williams et al., 2012) and the quantitative change causes thalassaemia.

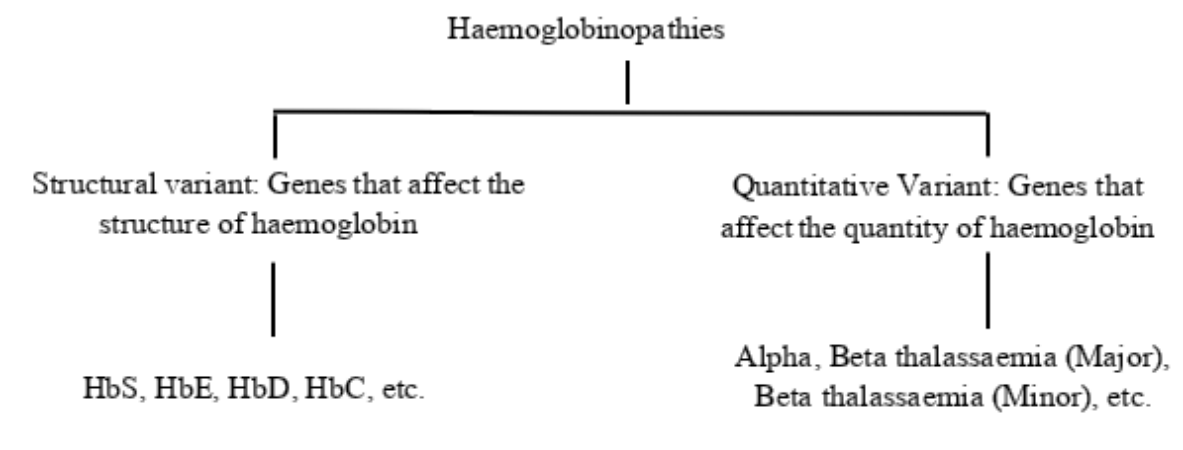

Figure 1.2: Classification of the haemoglobinopathies

Genetic defects that affect the production or structure of globin chains can cause haemoglobinopathies or thalassemia. Structural haemoglobin variants are due to point mutations in a β globin gene that produces a single amino acid substitution in a globin chain. Any change in the sequence of amino acid due to mutation or duplication is attributed to the formation of

abnormal haemoglobin, which gives rise to several forms of anaemia related to the chain. Mutations in the genes that code for the alpha or β chains potentially affect the biological function of the haemoglobin. The genetic defect may be due to the substitution of one amino acid for another (HbS, HbE) and the other abnormal haemoglobins, deletion of a portion of the amino acid sequence (Hb Gun Hill), abnormal hybridization between two chains (Hb Lepore), or abnormal elongation of the globin chain (Hb Constant Spring).

The Gene mutation for HbS affects the protein of the haemoglobin in red blood cells. This mutation results in an abnormal haemoglobin and causes red blood cells to become fragile and take the shape of sickle. The sickled cells of the haemoglobin cannot carry oxygen because of their shape causing ischemia, acute and chronic organ dysfunction involving the spleen, brain, lungs, and kidneys. Pain and swelling in joints of hands and feet was a frequent early occurrence of this diseases in infants and young children and result in aseptic necrosis of the small carpal, tarsal bones, femur head, and pelvic girdle. HbC was characterized by reduced RBC survival lifespan. However, haemolyses was not as severe as in sickle cell diseases. HbD was characterized by the substitution of glutamine for glutamic acid at the position of 121 of globin chain

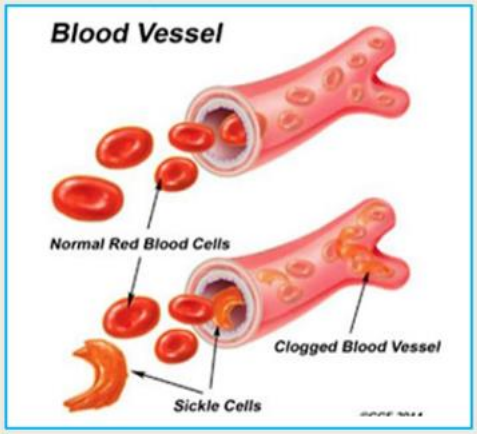

Figure 1.3: Normal and Sickle cell RBC.

Thalassaemia, derived from the two Greek words: Thalassa meaning the sea, i.e., the Mediterranean, and anaemia ("weak blood"), was also known as Mediterranean anaemia after its place of origin, was a quantitative defect of haemoglobin synthesis. The thalassemias are named according to the globin chain affected. Thus, alpha thalassemia was the result of decreased (α^{+}) or absent (α^{0}) production of alpha globin chains, and β thalassemia are likewise the result of decreased (β^{+}) or absent (β^{0}) production of β globin chains.

The homozygote state of haemoglobin leads to sickle cell diseases (HbSS) and was characterized by a point mutation involving the gene which encodes the haemoglobin subunit β (β) resulting from mutations in the β-globin gene (HBB) that code for haemoglobin subunits, having prototypical Mendelian single-gene disorders affecting the gene (Chatterjee, 1968, Thien, 2013). In India, anaemia was often neglected as a common type of nutritionally deficient health problem. It was supplemented with iron enriched diet or medicines to facilitate natural haemoglobin production in the body. Until or unless there are episodes of continuous deterioration in the health condition of the patient/individual, opportunities for proper diagnosis of severe anaemia, thus become one of the major national public health burdens, in terms of prevention and systematic management of haemoglobinopathies for decades.

Geographical distribution of Haemoglobinopathy and Thalassemia

Over 750 haemoglobin variations have been identified worldwide. Figure-1.4 shows the world distribution of sickle cell anaemia, HbS, HbE, HbD, HbC and β-thalassaemia.

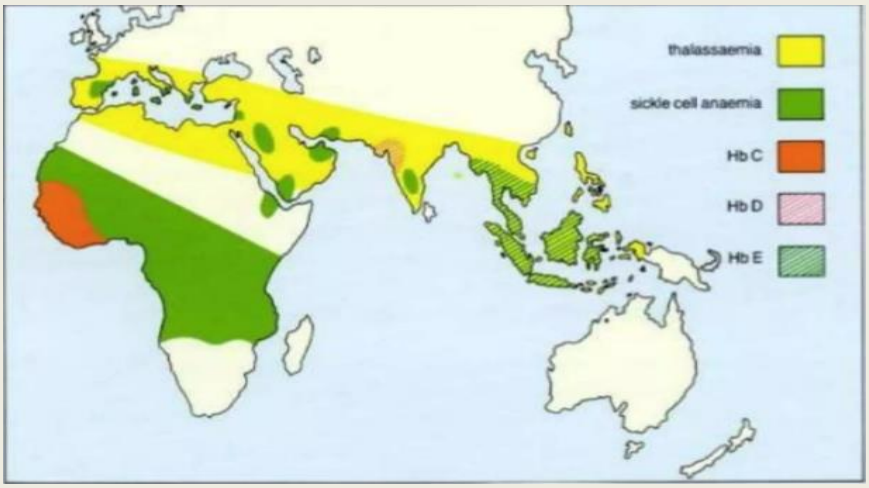

Figure 1.4: Geographical distribution of Haemoglobinopathies and Thalassemia
Source: https://www.slideshare.net/slideshow/thalassemiapptx-259238343/259238343

HbS occurs throughout sub-Saharan Africa and in small pockets in the Mediterranean region, the Middle East, and the Indian subcontinent. The frequency of the carrier for the gene, the sickle cell trait (SCT) was between 5 and 48 percent, distributed in different parts of India, predominantly in the central region followed by southern states and Odisha. Sickle Cell Diseases was a major health burden in the Vidarbh region of Central India including Chandrapur, Gadchiroli, Yavatmal, Wardha, Amravati, Gondia, Bhandara and Nagpur districts which have a huge tribal population and other weaker sections of society where carrier frequencies of the sickle gene can be as high as 25 to 30% in many of these communities. There are around 12000 Sickle Cell Diseases patients in Vidarbh region and over 3000 Sickle Cell Diseases patients in Chandrapur alone who require optimum management and there may be many more who are undiagnosed. Estimated numbers of sickle cell carriers in Vidarbh was approximately 4,00,000. β-thalassemia patients are also seen in this region.

The gene for HbC was prevalent in the African American population but with less frequency (2-3 percent) than that of the sickle cell gene. Hb D-Punjab (D-Los Angeles) was one of the abnormal haemoglobins found worldwide. It was present in many populations of Pakistan, and North-west India, especially at a high frequency in Punjab.

Haemoglobin E occurs widely throughout the north-eastern and eastern part of the Indian subcontinent, Bangladesh, Myanmar, and East and Southeast Asia. It occurs at varying frequencies but in some parts of Asia, notably, the northern parts of Thailand and Cambodia, called the "HbE triangle," up to 70 percent of the population are carriers (Fucharoen and Weatherall 2012). The mild forms of alpha thalassemia occur in the tropical belt stretching from sub-Saharan Africa through the Mediterranean regions and Middle East to the Indian subcontinent and the whole of east and southeast Asia. In this region, they occur at a frequency

of 10 percent to 25 percent, although in a few localized populations such as those of north and east India and Papua New Guinea, they are found in up to 20 percent to 80 percent (Yenchitsomanus, 1986).

The more severe forms of β thalassemia occur only in southeast Asia and in some of the Mediterranean Islands.

Although the haemoglobinopathies occur at particularly high frequencies in these tropical regions, they have been transported to most of the countries of the world by population migrations over many years. For example, the sickle cell gene have spread from the Caribbean Islands and in parts of North America to most countries. The same phenomenon also occurred in the case of thalassemia. However, no haemoglobin disorders are observed among the American Indian population, probably because haemoglobinopathy were not established in Asia at the time of the early population movements.

Mode of inheritance of haemoglobinopathies

Haemoglobinopathies are the case of an autosomal recessive pattern of inheritance. Sickle cell anaemia or Thalassaemia was a completely an autosomal genetic disease. The recessive genes of the parents play an important role in the genetic pattern of the diseases in a child.

If both the parents are carriers of the abnormal gene i.e., their genotypes of genes are β^0/β^+ and the couple has four children, there will be the possibility of one child being normal, two children being carrier or trait and one child being patient. For example, in case of thalassaemia, β^0/β^0 was thalassaemia patient, β^+/β^0 was thalassaemia trait and $\beta^+\beta^+$ was normal (Figure 1.4). In case one of the parents was normal and the other parent was patient and if they have four children, the phenotype will be $\beta+/\beta^0$ for all, so all will be thalassaemia trait. If one parent was normal and the other one was carrier, then two of the children will be thalassaemia carriers and two of them will be normal children.

HbE β-thalassemia may be inherited from a parent who was homozygous for haemoglobin E and a partner who was heterozygous for β-thalassemia. Very rarely, the condition was inherited from a parent who also has HbE β-thalassemia, and that partner was a carrier for either HbE or β-thalassemia. An individual may inherit an HbS gene from one parent and an HbC gene from the other resulting in HbSC diseases. The clinical severity of this condition was intermediate between that of sickle cell diseases and HbC diseases, except that visual damage due to retinal vascular lesions was characteristically worse in HbSC diseases than in SCD.

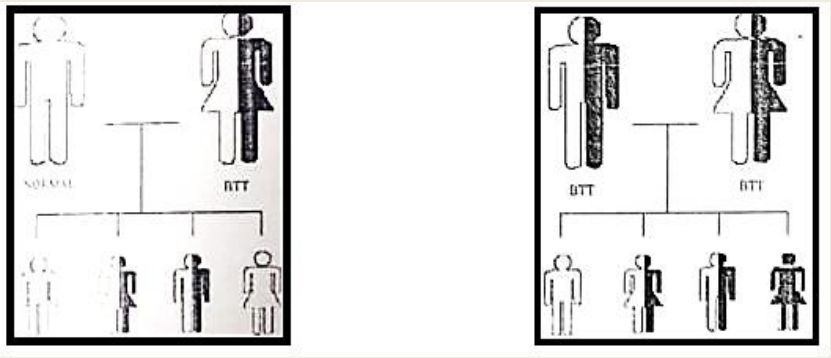

Figure 1.5: Mode of inheritance between normal and carrier and between two carriers

Review of literature

Haemoglobinopathy as a genetic disease was first recognized by Thomas Cooley after he described thalassemia as a disorder in patients of Italian origin in 1925. James Herrick, a physician first defined the characteristic sickle shaped red cells in a medical student from Grenada in 1910. The Indian subcontinent was a hub of sickle cell diseases (SCD), β-thalassaemia and various abnormal haemoglobins because of high consanguinity, caste and area endogamy. Numerous studies in Indian context shows the prevalence of the diseases. Sickle cell haemoglobin (HbS) was first detected in Nilgiri Hills in south India by Lehman and Cutbush, in 1952. In the same year, Dunlop and Mazumder also reported the presence of sickle haemoglobin in the tea garden workers of Upper Assam who were migrant labourers from tribal groups in Bihar and Odisha (Dunlop and Mazumder, 1952). Linus Pauling and his co-workers revealed that sickle haemoglobin (HbS) had an improved electrophoretic mobility, and they were the first to define that it was a molecular disease in 1949. Allison (1954) working on east African populations put forward sickle cell - malaria hypothesis that the individuals with sickle cell trait (HbAS) were less likely suffered with malarial infection than the healthy individual with normal haemoglobin. Thus, it became a good example of balanced polymorphism as the malarial parasites confer protective mechanism to the affected red cells in heterozygote condition than in the homozygote state. Natural selection should therefore have purged this mutation from human populations (Haldane,1949). A few years later in 1957, Vernon Ingram discovered that sickle haemoglobin resulted from a single amino acid substitution in the haemoglobin molecule. Some communities exhibit the higher incidences of the diseases, what determines a major public health problem (Balgir, 2000). This alteration in a single DNA base leads to a cascade of physiological consequences that can affect multiple organs and systems (Rees et al., 2010). Polymerization of the two-mutant sickle β-globin subunits leads to erythrocytes assuming a crescent or sickled shape, thus the designation of sickle cell diseases (SCD) (Tebbi, 2022). From various screening carried out in India, it was noted that the average carrier prevalence rate of β-thalassaemia was 4-9 percent in India (Urade, 2012; Colah and Gorakshakar, 2014; National Health Mission, 2016). The expected annual number of affected births can be estimated as 0.5/1000 live births (Sinha et al., 2020). In 2016, an estimated 120,000 people with SCD were born in India and an equal number were born with the HbS-β-thalassaemia genotype that contributed to the SCD phenotype (Rees and Brouse, 2016). Neonates with the Sickle Cell (SS) genotype would account for 1.5/1000 live births. A study proposes that in the decade 2017–2026, there would be 37,5000 HbSS neonates born (Sinha et al., 2020).

There are five different haplotypes of sickle cell diseases across the world, namely, Senegal, Bantu, Benin, Arab Indian and Cameroon. In India, although many more people are affected by SCD than thalassaemia major, the causative mutation mainly was linked to the Arab Indian haplotype, which was clinically much less severe than the African haplotypes (Colah et al., 2018). Buchi (1955) stated that the sickle cell was not necessarily a tribal trait. Sickle cell diseases was not only confined to the lower castes or tribal groups (Balgir 2005; Urade 2012) but it was present among other communities in varying frequency. According to Labie et al. (1989), the sickle cell diseases has got unicentric origin of the mutation in India.

There was diversity within the haplotypes of sickle cell mutation in various ethnic groups which indicates that the mutation might have occurred independently in various ethnic groups during human evolution. Contrary to the findings from other parts of the country that the gene HbS was confined to scheduled tribes, it was found that HbS was prevalent even in different castes and communities in India.

The HbS gene was prevalent among the general caste including Brahmins (Sinha, et al. 2004; Balgir 2006; Urade 2012b). It was evident from the literature that the several ethnic groups with varied genetic elements have been assimilated into the mainstream, resulting in population diversity with the passage of time (Russel and Lal, 1916). This situation leads to the parallel divergence of subgroups of the same community that the one group with the deleterious gene emerged while another group of the same ethnic elements evolved unaffected during time. The subdivisions of the population of India by geographic, linguistic, religious caste and other barriers has resulted in the existence and perpetuation of thousands of distinct highly inbred communities (Brittenham, 1988).

This remarkable genetic heterogeneity was a distinctive feature of the Indian population which accounts for uneven and variable distribution of the haemoglobinopathies (Iswad and Nayak, 1984). However, Balgir (2005, 2006) in Odissa reported higher prevalence of sickle cell among the general castes (0.3 - 20.7) followed by scheduled castes (0- 8.9) and scheduled tribes (0 - 5.5).The prevalence of sickle cell diseases (SCD) was high in tribal and scheduled castes, but it was highest among other castes groups of Kurmi (55) and Teli (53) belong to backward castes of Chhattisgarh (Patra et al. 2010). WHO (2006) has reported an estimate of about 20-25 million homozygous individuals for sickle cell diseases worldwide of which 5-10 million are in India (Sergeant, 2006).

Since then, many communities have been screened and the sickle cell gene was shown to be prevalent among three socio-economically disadvantaged ethnic groups, the scheduled tribes, scheduled castes and other backward classes in India (Kaur, 1997; Kate, 2002; Patra et al., 2011; Urade, 2012; Colah, 2014).

The prevalence rate across India varies from region to region amongst many Indian ethnic populations. In India, HbS gene (HbSS for diseases and HbAS for carrier) was mostly confined to tribes in central and south India and the frequency ranges from 5 to 35 percent (Bhatia and Rao, 1987). In Central India study on sickle cell anaemia has been carried out mostly on tribal groups and very few on castes and other populations. The prevalence rate of HbS and Hbβ Thal in different ethnic groups ranges from 0–20 percent (Urade. 2012). Published data suggest that some 50 percent of all homozygotes for HbS are born in the states of Karnataka, Tamil Nadu and Maharashtra (Piel et al. 2013; Colah et al. 2014) where consanguineous marriages are widely favoured (Bittles, 2012). High rates are recorded in the western, eastern and central states of Gujarat, Odisha, Madhya Pradesh and Chhattisgarh (NHM, 2016). Being a neglected health problem, the overall research on sickle cell diseases (SCD) has also been scarce in India (Chakraborty and Williams, 2013; Serjeant et al., 2016). The prevalence rate across India varies from region to region amongst many Indian ethnic populations. In India, HbS gene (HbSS for diseases and HbAS for carrier) was mostly confined to tribes in central and south India and the frequency ranges from 5 to 35 percent (Bhatia and Rao, 1987). In Central India study on sickle cell anaemia has been carried out mostly on tribal groups and very few on castes and other populations.

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Furthermore, research on SCD remains fragmented across disciplinary areas, largely from the biomedical disciplines (especially haematology, pathology and genetics), with limited application of a broad, interdependent and dynamic health systems perspective (Raman et al., 2021). Moreover, there has been minimum effort in generating information on the ethnic distribution of the haemoglobinopathies especially in large, scattered population with heterogeneous composition.

Carrier screening as a part of the prevention curriculum was a prerequisite for any national haemoglobinopathies control program. In India, newborn screening programmes for sickle cell disorders among tribal and non-tribal populations have only initiated during the last decade in south Gujarat, Maharashtra, Chhattisgarh, Odisha and Madhya Pradesh (Mohanty,2012; Panigrahi et al., 2012; Jain et al., 2012; Italia et al.,2015). Most of the tribal populations where sickle cell diseases were common rely on the primary health care facilities in rural and often remote areas. The Indian government has elaborated the strategic roadmap for screening around 70 million people under the age of 40 years for SCD patients in 200 districts of 17 states in three years. And this screening was to eliminate the diseases in the next 25 years (Babu et al., 2022). The α-thalassaemia's are more common worldwide compared to the β-thalassaemia's (Weatherall, 2018). Having a load of carrier of this gene varies about 5-50 percent (Kate, 2008). The average prevalence of β-thalassaemia carriers was 3–4, which translates to 35–45 million carriers. Estimates indicate that there would be around 100 000 patients with a β-thalassaemia syndrome, but the exact numbers are not known because of the absence of national registries (Verma et al., 2011).

Extensive studies have provided data on haemoglobinopathies; these include multicentre studies covering different states conducted by the Indian Council of Medical Research.

Chatterjee et al. (1957) reported the first case of β-thalassaemia and Hb E diseases in India. Due to the heterogeneous and diverse population in India, it was not surprising that there was a wide range of prevalence of thalassaemia in different states. In India, every year 10,000 children are being born with thalassemia which approximately accounts for 10 percent of the total world incidence of thalassemia-affected children (Sengupta, 2007) and one in eight of thalassemia carriers live in India.

The prevalence of thalassemia ranges between 0.6 and 15 across south India (Shantaram, 2016). In the eastern part of India, the influence of HbE mutations also plays a part in defining the clinical phenotype of the thalassaemia. In a multicentre study involving 56780 individuals from six major cities (Bengaluru, Kolkata, Dibrugarh, Ludhiana, Mumbai and Vadodara) between 2000 and 2005, it was estimated that the prevalence of Hb disorders ranged between 3.1 percent and 31.8 percent.

The type of thalassaemia also varied with β-thalassaemia being the predominant diseases seen in Bengaluru (overall 3.1 percent; β-thalassaemia 2.16 percent), Ludhiana (overall 5.2 percent; β-thalassaemia 3.96 percent), Mumbai (overall 3.48 percent; β-thalassaemia 2.55 percent) and Vadodara (overall 3.38 percent; β-thalassaemia 2.68 percent). While in Kolkata, both β-thalassaemia trait and HbE diseases were equally prevalent (overall 8.3 percent; β-thalassaemia 3.64 percent; HbE 3.92 percent) and in Dibrugarh, it was predominantly HbE diseases (overall 31.8 percent; HbE diseases 29.2 percent). In another study involving 1291 subjects in western Maharashtra, the incidence of Hb disorders was 11.43 percent with the main diseases being β-thalassaemia major. In central Maharashtra the highest prevalence of β-thalassaemia found to be more than 9 percent among the Sindhi population (Urade, 2012).

An analyses of 1015 subjects with anaemia in Odisha between 1994 and 2003 brought out a prevalence of sickle cell trait (29.8%), sickle cell diseases (7.5%), sickle cell-β-thalassemia (1.7%), β-thalassemia trait (18.2%), thalassemia major (5.3%), and other haemoglobinopathies (Balgir, 2005). A higher frequency has been observed in certain communities, such as Sindhi, Punjabi, Gujarati, Bengali, Mahar, Koli, Saraswat, Lohana and Gaur (Balgir, 2006). Within each state, the prevalence varies based on the presence of ethnic groups (Madan et al., 2010). At present, the complete and only treatment available for thalassemia major was bone marrow transplantation, which only a few patients can afford. For supportive care and management of a child with thalassemia major nearly 100,000–250,000 INR/year was required depending on the age and presence of complications (Petrou, 2010; Thiyagarajan, 2018). B-thalassemia might be prevented by the identification of carrier, prenatal diagnosis, and genetic counselling (Origa & Comitini, 2019). Genetic counselling offers the evidence for the risk of carriers of both parents and risk in offspring. Community-level screening among the pre-marital group and detection of carrier status was the way to control the diseases among the next generation. Early screening allows adequate time for work-up and counselling of the couples. Antenatal screening of pregnant women also helps in prevention of thalassaemia (Richard et al., 1995). Lack of awareness due to illiteracy (MHFW, 2018) and insufficient flow of resources contributes to the wider spread of the diseases; which in turn leads to an increase in burden and its after-effects (Akhlaghpoor, 2006; Behdani et al., 2015). It was expected that the global economic burden of the haemoglobinopathies on public health will increase over the coming decades (Weatherall, 2010), 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 haemoglobinopathies in India.

We understand that the detection and counselling 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, Iran where there has been an attempt to address this serious health problem taken 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.

Challenging situation in India

Haemoglobinopathy was one of the major public health issues in India. Timely identification of these disorders was of paramount importance to prevent clinically severe haemoglobinopathy as well as for epidemiologic purposes. It was now understood the central, western and eastern part of the country harbour the disorders in higher frequencies compared to the rest of the country (Balgir 2000). With increasing retrospective model of screening, large number of cases can be detected which help to reach the medical management facilities to the diagnosed patients in India. Proper medical care minimizes the clinical severity and lengthen the life span. Though there are number of undetected and untreated cases which can be identified by population based prospective mode of screening.

WHO recognizes comprehensive approaches (improve curative services, establish prenatal diagnosis, develop carrier detection and counselling, improve education) in community control of haemoglobinopathies (Angastiniotis et al., 1995). The problem with many of the current screening and counselling programs was that they don't consider the recipient's socio-psychological background during exerting the counselling. In majority of cases, it has been seen that after the reporting of cases identified as carrier of any haemoglobinopathies, there has been a notable stigma developing during marriage alliances or decisions of pregnancies. The available alternatives are too costly (e.g., postnatal diagnosis PND) or misleading (e.g., decision taken by the couples not to go for pregnancies). Widespread dissemination of information on thalassaemia and sickle-cell disorder, and possible strategies for their control was much needed to decrease the burden of the disorder. It was understood that the detection and counselling 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.

However, the greatest challenge was to introduce these approaches in large communities comprising of a mosaic of ethnic groups at various level of risk to haemoglobinopathies like India (WHO, 1983). Studies undertaken on haemoglobinopathies in West Bengal have reported higher frequency of β thalassaemia (Bandopadhyay et al 1999), alpha thalassaemia (Nadkarni, 2008), structural variant haemoglobins (Das et al 2000, Das et al 1991, Deka et al 1988) and anaemia to be prominently present. However, less was known about the ethnic diversity of the haemoglobinopathies of people of West Bengal as well as the distribution of mutations among them with an attempt to ascertain the possible origin of them. The present program was initiated in this part of the country to address these issues with the major aim of mass screening and generating public awareness.

Thus, the goals of medical genetic services should be to help these people with a genetic disadvantage and their families to have access to quality care as well as social and genetic counselling support to make informed choices for reproduction to have healthy children with the availability of prevention programmes when needed (Colah et al., 2015).

Significance of Study

Haemoglobinopathy was a major hereditary disorder affecting the blood, leading to high mortality rates in childhood. It affects both tribal and non-tribal communities in India. Despite increased medical management facilities and retrospective screening models, the number of cases with haemoglobinopathies continues to grow with minimal clinical severity. However, there was a constant rise in untreated and undetected cases. Population-based prospective screening was needed to accurately detect the exact number of cases. This study was crucial for improving public health in India such as:

1. To determine prevalence of haemoglobinopathies in India and study the different variants at different age group/ sex of peoples irrespective of caste and community in different states of India, using HB electrophoresis, CBC, HPLC testing system. and molecular detection.
2. The basic aim, besides screening for the dreaded genetic disorder, also includes generating relevant awareness at the community level.
3. To observe whether carrier for the HbS, HbE and β-thalassaemia was community specific and/or village specific.
4. To identify more carrier form index cases by inductive screening method and cascade screening, and to conduct awareness campaign and genetic counselling of the thalassaemia carriers.
5. Molecular characterisation of BTT.

Limitation of the Study

The problem with many of the current screening and counselling programs was that they did not consider the recipient's socio-psychological background during counselling. In most cases, it has been seen that after the reporting of cases identified as carriers of any haemoglobinopathies, there has been a notable stigma developing during marriage alliances or decisions of pregnancies. The available alternatives are either too costly for example, pre-natal diagnostics (PND) or misleading decision taken by the couples not to go for pregnancies. AnSI understand that the detection and counselling 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 (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 was to introduce these approaches in large communities comprising a mosaic of ethnic groups at various level of risk to haemoglobinopathies like India (WHO, 1983.

Methodology

Anthropological Survey of India conducted a large-scale multi-phased mass-screening program under the National Project, "Community Genetics, Health and Bio-Cultural Diversity' from 2005 to 2015 among various ethnic groups and tribal populations inhabiting different districts of Central India, Eastern India, North-Eastern India, and Andaman and Nicobar Islands. The study was approved by the Institutional Human Ethics Committee (IHEC), Anthropological Survey of India, in 2005.

Operation Strategy:

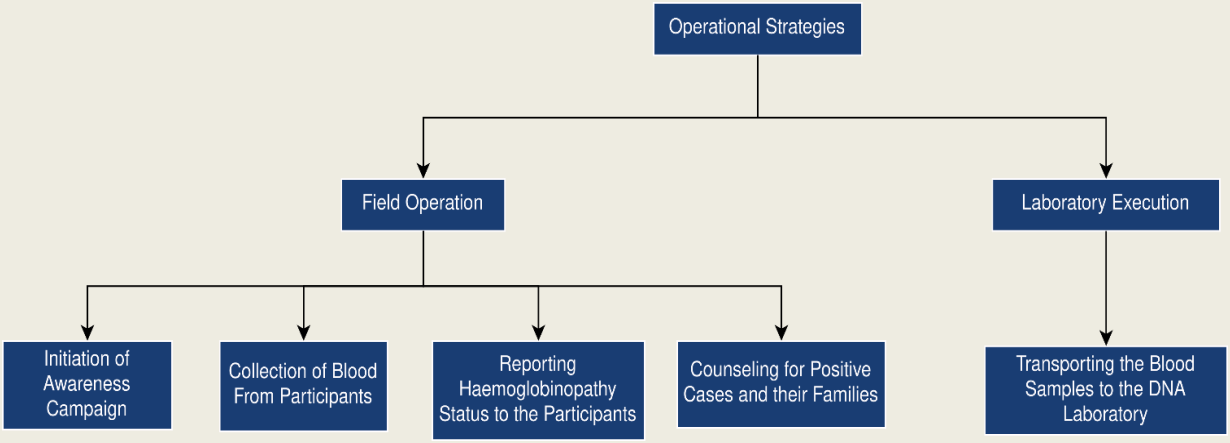


Figure 1.6**:** Operational strategies of the study

Initiation

On initiation of the project, areas were selected for screening in places where the disorders were at higher frequencies compared to the rest of the country as suggested by earlier records (Balgir 2000). The areas vulnerable to sickle cell in central India, thalassaemia in eastern India, HBE in Northeastern India, sickle cell and thalassaemia in Southern India and Andaman and Nicobar Islands were selected as study area with high-risk populations. A series of meetings were conducted in three tires covering the block level administrators, executives of local bodies, gram panchayat members and finally mass meetings at the village level with the common target groups.

School teachers were also involved during the village level meetings. Schools and community centres were chosen for the arrangement of the camps. Adequate interactions were established before the camps to get the target groups aware of the outcomes and the benefits of haemoglobinopathy prevention programs.

During this phase, a preliminary survey was also conducted to generate information about the general demographic profile, family type and size, general health problems, history of haemoglobinopathies in the families and level of education of two generations including the target groups. A written consent was asked for before registering the name of the subject in the list of participants.

Target group specified for screening of carrier status.

- Unrelated individuals in premarital age group–for mass screening to detect haemoglobinopathy.
- Youth – to increase the awareness on the prevention of the disorder.
- Affected families- to encourage voluntary screening for thalassemia among the relatives (cascade screening).
- General community- to reduce myths and misconceptions.

The target areas screened was where the intensity of the gene was high and where the individuals could not afford to be tested. The major objectives rested on the understanding of the ethnic and cultural diversity of the participants in the ongoing Community Genetics Extension Program. Simultaneously prime objective of establishing a central protocol for laboratory diagnosis of haemoglobinopathies at community level was also targeted. The protocol involved infrastructural development at the selected central laboratory and developing peripheral centres. The infrastructure includes facilities of field tests, haematology, biochemistry, and molecular characterization of haemoglobinopathies up to the β-globin gene sequencing. The detected cases were followed up for the advanced understanding of the possible origin of the mutations and the level of ethnic admixture.

The blood samples collected from participants included screening and confirmatory tests. The summary of the execution was given in Figure 1.7.

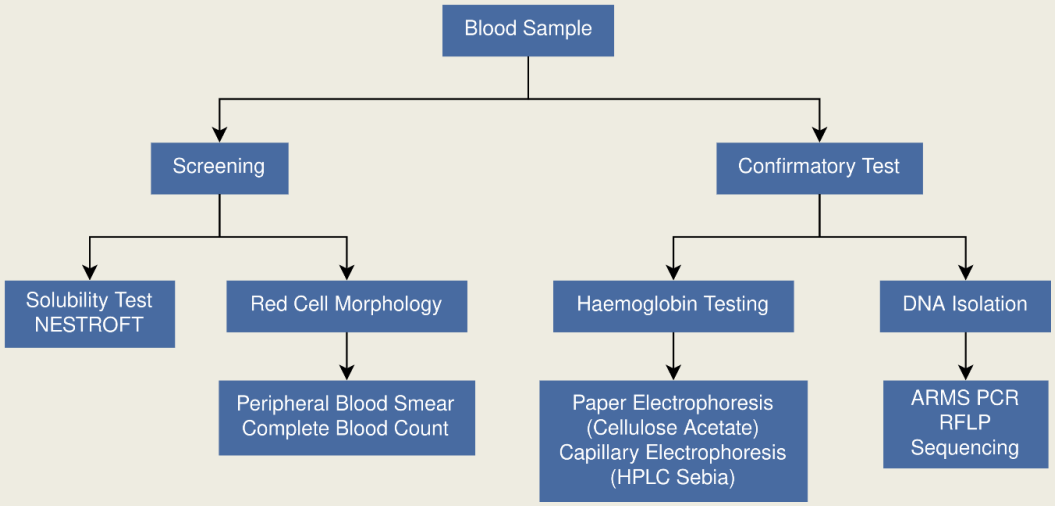


Figure 1.7: Laboratory execution for detection of haemoglobinopathy.

Intravenous blood of suspected individuals was taken in ethylenediamine tetra acetic acid vials (K_3 EDTA vacutainers, BD, Franklin Lakes, USA), following the standard procedure. Approved consents were obtained from everyone before the collection of venous blood samples. For the participants below the age of 18 years (minor), the consents were obtained either from parents or Principals of the school wherever it was necessary. Data collection was adhered to purposive-cum-simple random technique of sampling to meet the objectives of the projects.

The collected blood samples were brought back to the central laboratory of respective Central, Eastern, and North-eastern Regional Centre and ANRC. The following tests were carried out.
Peripheral blood (PB) smears
PB smears provide relevant clues to initial detection by identifying morphological abnormalities of erythrocytes in β-thalassaemia or sickle cell. The number of blood cells was determined by using light microscope at 1000-fold magnification (HPF-high power fields).

Solubility Test and NESTROFT

Solubility test (K_2HPO_4, KH_2PO_4, $Na_2S_2O_5$, and Saponin) was based on the relative insolubility of haemoglobin S in the reduced state in high phosphate buffer solution (metabisulfite, a reducing agent). When whole blood was mixed with the reducing agent, the haemoglobin S forms liquid crystals and give a cloudy appearance to the phosphate buffer solution. A transparent solution was seen with normal or other haemoglobins that are more soluble in the reducing agent. A positive result was indicated by a turbid suspension through which the ruled lines are not visible (Figure:1.8) A negative result was indicated by a transparent suspension through which the ruled lines are visible.

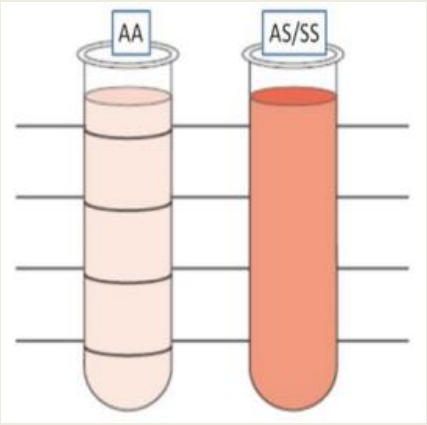


Figure 1.8: Result of Solubility Test/NESTROFT

NESTROFT (Naked eye single tube red cell osmotic fragility test) was used to assess osmotic fragility of red cells at a single concentration of buffered saline (0.36% in single tube) visually, to screen sickle cell trait, HbE or β thalassaemia was performed on the spot using the prescribed method (Kattamis et al 1981). A volume of 20 µl of peripheral blood samples were drawn from finger prick and mixed in 2 ml (i.e., 1:1000 ratio) solutions for solubility test and NESTROFT test separately and mixed thoroughly. Results were noted after 3 minutes for HbS and for HbE and haemoglobin β-variant after 25-30 minutes. Samples with turbidity and opaque solutions were considered suspected cases. 2ml intravenous blood samples from suspected individuals were brought to DNA lab for further analysis.

Alkaline or paper electrophoresis

This procedure was useful in picking up suspected cases for haemoglobinopathies. The detections of heterozygote and homozygote state of β thalassaemia and other variant haemoglobins are confirmed by running the hemolysate on the alkaline cellulose-acetate membrane. Dry Cellulose Acetate Membrane (Sartorius, Germany) was used for electrophoresis. The strips were carefully dipped in TEB buffer at pH 8.4 for about three hours, soaked between Whatman#1 filter paper was placed on the bridge of the tank connected to the buffer tray. 200 µl whole blood was used to prepare for lysate which were then washed with CCL_4. All the lysates along with control were subjected to electrophoresis at 80 Volts for about 25-30 minutes to get clear band following which the results were noted down.

Complete blood count (CBC)

The blood samples were subjected to complete blood count for haematological parameters like white blood corpuscles (WBC), red blood corpuscles (RBC), haemoglobin content (Hb), haematocrit (HCT), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (Plt), lymphocytes (Lym), monocytes (MO), granulocyte (GR), red cell distribution width (RDW), and mean platelet volume (MPV). The mean value of all the above-mentioned parameters was considered for interpretation in the present study.

Suspected samples for detection thalassaemia were subjected to osmotic fragility test (OFT) and for sickle cell, cellulose electrophoresis was done. After screening for CBC, samples were subjected to further screening through cut-off values indicating the possibility of heterozygosity for haemoglobinopathy or thalassaemia. This includes: MCV < 78 femtoliters (fl) and MCH < 27 picogram (pg). Evaluation of the blood counts in samples >24 hours was made with caution as the red cells increase in size leading to falsely raised MCV values. Majority of heterozygotes for β thalassaemia, whether it be β -ve or β +ve, have hypochromic (MCH 19-23 pg) and microcytic (MCV 62-75 fl) red cells.

Capillary electrophoresis of haemoglobin

After the haematological test, the hemolysates were prepared and subjected to haemoglobin testing system (Variant Machine, Bio-Rad, HPLC or Sebia capillary electrophoresis). Based on positions of the peak in the given retention time or zone of the capillary electrophoresis viz. peak at Zone-9 for normal, Zone-5 for HbS and Zone-3 for Hb β-thalassaemia and HbE, the results were noted down accordingly for sickle cell carrier, β-thalassaemia minor and other disorders. For the determination of thalassaemia status, the cut-off point of 3.5 percent at Zone-3 was considered positive for Hb-βthal but for HbS only a position of peak at a particular zone was considered as positive. An elevated HbA_2 level in the range of 3.6-5.5 percent deviation from the typical haematological phenotype of the β-thalassaemia trait includes:

- Reduced MCV and MCH with borderline or normal HbA_2 levels indicates iron deficiency, α-thalassaemia, heterozygosity for mild β-thalassaemia mutations, heterozygosity for delta-β-thalassaemia (δβ-thal)
- Borderline/normal MCV and MCH values with elevated HbA_2 indicates co-inheritance of α and β-thalassaemia.
- Normal or reduced red cell indices with normal HbA_2 but elevated HbF values which indicates heterozygous δβ-thal or Hereditary persistence of fetal haemoglobin (HPFH). Borderline HbA_2 levels (3.4-3.7 percent) in many normal individuals probably form the upper end of the normal range of HbA_2 values.
- The percentage of HbA_2 value more than 3.5 was taken as a β thalassaemia carrier status. The percentage of HbA_2 value was in the range of 23.2 to 35.5 a special feature of chromatogram which shows an addition of HbA_2 HbE window for HbE carrier status and a sickle window for HbAS /SS status.

Molecular characterisation

For molecular characterization, DNA was extracted of all the positive cases of HbS, HbE and Hb-βthal.

DNA isolation

A total of 2 ml of venous blood was used to extract DNA by salt saturation, phenol chloroform procedure (Miller et al 1998). Genomic DNA extraction was done from white blood cells using standard protocol. DNA extraction by phenol chloroform method

All reagents used for DNA extraction were molecular grade, DNase free and autoclaved MilliQ water (Millipore Life Sciences, USA) was used in this work.
Details of the buffers and reagents used for DNA extraction are given below.

RBC lysis buffer (pH 8.0)
0.5 M Magnesium chloride 5 ml
0.5 M Tris HCl 10 ml
0.5 M Potassium chloride .5 ml
Made up to 990 ml with MilliQ water, 10 ml Triton-X and made up to 1000 ml.

Digestion buffer (pH 8.0)
1 M Tris HCl 40 ml
1 M Sodium chloride 15 ml
0.5 M EDTA (Na salt) 10 ml
Made upto 95 ml with MilliQ water,5ml of 20% SDS added at room temperature.

Tris EDTA buffer (pH 8.0)
1M Tris HCl (pH 8.0) 2 ml
0.5 M EDTA 4 ml
Made up to 200 ml with MilliQ water.

Procedure

1. To the blood sample, 2 volumes of RBC lysis buffer were added and mixed gently by inverting the tube till the solution became transparent.
2. Centrifuged at 2500 rpm for 10 minutes to obtain a pellet. The supernatant containing haemolyzed RBCs was discarded carefully.
3. The pellet was re-suspended in RBC lysis buffer (equal to initial blood volume) and tapped gently to disturb the pallet.
4. Centrifuged at 2500 rpm for 10 minutes and the supernatant was discarded to obtain clear white pallet. This procedure was repeated until the pellet was free of RBCs.
5. The pellet was disturbed thoroughly in digestion buffer (500 micro litre) was added.
6. Proteinase K (Sigma Aldrich, India) was added to a final concentration of 10 µg/ml and sodium dodecyl sulphate (SDS) was added to make 1% concentration in the final solution. It was mixed thoroughly and gently by inverting the tube for 3-4minutes till the solution became viscous and thereafter incubated for 3-4 hours at 56°C for proper digestion of proteins.
7. When solution became clear 5 M sodium perchlorate ($NaClO_4$) was added (1/4th the volume of digestion buffer) and mixed gently for 3-4 minutes.
8. Phenol (Tris saturated), chloroform and iso-amyl alcohol in 25:24:1 ratio was added in equal volume to mixture of digestion buffer and 5 M $NaClO_4$. After mixing for 1 minute centrifuged at 4000 rpm for 15 minutes at 4°C.
9. Aqueous layer was transferred carefully into another sterile polypropylene centrifuge tube using a broad mouth tip.

10. Equal amount of chloroform and iso amyl alcohol in 24:1 ratio was added to the transferred supernatant and mixed gently for 3-4 minutes and centrifuged at 4000 rpm for 15 minutes at 4°C. After centrifugation the aqueous phase was transferred to a freshly labelled tube.
11. Exactly double the volume of chilled absolute alcohol was added and mixed gently by inverting the tube to precipitate the DNA.
12. DNA lump was pooled out into a fresh labelled 1.5 ml tube to wash the DNA twice with 70% ethyl alcohol.
13. The pellet was dried at room temperature properly to ensure that whole alcohol was evaporated.
14. DNA pellet was dissolved in 200 µl of Tris EDTA (TE) buffer, for optimum dissolution tubes were kept in water bath at 56oC for 20 to 30 minutes. Dissolved DNA samples were stored at-80oC.

DNA quality check by electrophoresis

Quantity and quality of extracted DNA was checked by gel electrophoresis. Electrophoretic analyses of DNA using agarose gels can confirm DNA integrity. The quantification of the isolated DNA was done by Parkin Elmar Spectrophotometer and checked by Agarose gel using Bio-Rad gel documentation system.

Quantification of DNA- The extracted DNA was quantified by the spectrophotometer followed by checking in 1percent agarose gel (Maniatis et, al., 1989).

Tris acetate EDTA (TAE) buffer 20X (pH 8.0)

Tris	48.4 g
Acetic Acid	11.402 ml
0.5M EDTA	20 ml

Dissolved in 1 Lt MilliQ water. 50 ml of 20X buffer was made up to 1000 ml with MilliQ water to obtain 1X TAE buffer.

Loading dye (stock)

Bromophenol blue	25 mg
Xylene cyanol	25 mg

Dissolved in 10 ml of MilliQ water. 5 ml of 40% sucrose solution was added to 1 ml of loading dye stock to make working dilution.

Ethidium Bromide solution (6%)

0.6 g Ethidium bromide was dissolved in 2 ml MilliQ water and made up to 10 ml.

Spectrophotometer

Absorption spectrum of DNA was between 260 and 280nm. At 260nm, an absorbance of 1.00 OD, measured in a cuvette with 1 cm path length, was indicative that concentration of DNA was approximately 50µg/ml.

Concentration of DNA (µg/µl) = OD at 260nm x dilution factor x 50µg/µl.

The ratio of absorbance at 260 to 280nm indicates the purity of the sample. This ratio of DNA solutions should range from 1.7 to 1.8. The presence of impurities like proteins or phenol tends to decrease this ratio. 5µl of the DNA solution was taken and diluted 100 times by adding 995µl of DDW and mixed well. OD readings were taken in a preset spectrophotometer at 260nm and 280nm using DDW as blank. The concentration of DNA was then accordingly calculated. With the help of concentration of DNA (ng/µl), the respective volume of DNA was taken and diluted with TE solution, so that the conc. becomes 10ng/µl DNA. For example, if the concentration of DNA was 750ng/µl, 5µl of DNA was taken from stock and 370µl of TE solution was added to it.

Gel electrophoresis

Agarose gel electrophoresis was an efficient technique to separate DNA molecule according to their molecular weights in the same manner as a sieve. 0.8 gm of agarose was dissolved in 100 ml 0.5X TAE buffer in a 250ml conical flask and was boiled to dissolve agarose completely. 0.7µl Ethidium bromide was added from stock solution to make a final concentration of 0.5µg/ml. Gel was cooled down to 60°C, poured onto a gel tray and was allowed to set. A standard DNA sample of known concentration was also loaded along with the samples to quantify DNA, and electrophoresis was carried out at a constant voltage of 80V.After 30 Min. of run halfway the gel was observed under ultraviolet light of UV trans-illuminator and photographed.

Molecular detection

ARMS-PCR was performed using appropriate oligo-specific primers to detect known mutations and confirm cases of HbE, HbS and β-thalassaemia detected by HPLC. This technique uses three oligo-nucleotide primers, and the length of normal and mutant primers are similar. GeneAmp PCR system 9700 of Applied Bio-systems, USA, was used to run the PCR program. The oligonucleotide primers (Sigma-Aldrich, USA) used for detection of the mutant allele were as prescribed protocol. ARMS-PCR method (Varawalla et. al., 1991, Bravo et. al., 1999) and Sequencing through 3730 DNA Analyser. GeneAmp PCR system 9700 of Applied Biosystems, USA were used to run the PCR program. The oligonucleotide primers (Sigma-Aldrich, USA) used for detection of the mutant allele were as prescribed ().
Preparation of DNA working dilutions
100 µl of DNA working dilutions were prepared at a concentration of 50 µg/µl by dissolving required amount of stock DNA sample in MilliQ water. The uniformity of the samples was checked by performing electrophoresis on a 1% agarose gel.

Procedure

Agarose gel was prepared by adding required quantity of agarose (1%) to 1X TAE buffer and mixed well. The mixture was heated in microwave oven until it became clear, and care was taken to avoid over boiling and evaporation. The mixture was cooled to ~50^{o}C and 2 µl of 6% ethidium bromide was added. The entire mixture was poured into a tray in which a comb was fixed for the purpose of making wells.

After gel formation the tray was placed in buffer tank containing 1X TAE buffer for submerged gel electrophoresis and the comb was removed with care to avoid rupture of wells. 1 µl of each DNA sample was mixed with 1 µl of loading dye and the mixture was loaded into the wells. Gel was subjected to electrophoresis at 100 V for 20 minutes and visualized using gel documentation system (Syngene, UK) for recording of the results.

Primers

For complete β globin gene sequence was using standard 3 pairs of both forward and reverse primers (Table 1)

Table 1.1: Primers used in the HBB sequencing.

Oligo Name	Sequence	Position on the β globin gene
RDB1	GTACGGCTGTCATCACTTAGACTTCA	IVS1
PCO6	TCATTCGTCTGTTTCCCATT	
IVS2F	TCTTTCCCCTTCTTTTCTAT	IVSII
IVS2R	CCTCTTACATCAGTTACAAT	
TH6F	CAATGTATCATGCCTCTTTGCACC	Exon 3
TH7R	GAGTCAAGGCTGAGAGATGCAGGA	

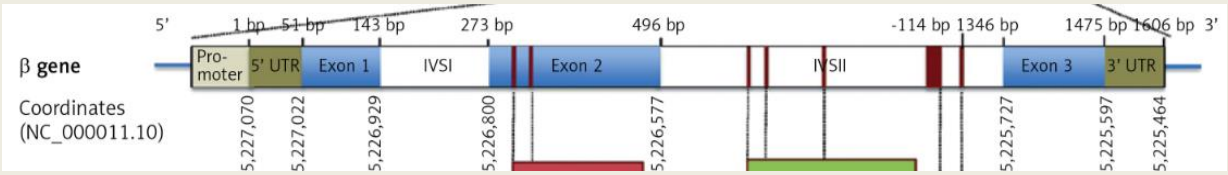


Figure 1.9 Beta globin gene

There are 3 basic steps of a PCR reaction:

Denaturation- To amplify DNA, the two strands of the template DNA first have to be separated. This occurs by heating the dsDNA template to a point where the hydrogen bonds break between the base pairs. This results in the separation of the two DNA strands.

Annealing- The temperature was then dropped to a range in which the forward and reverse primers are stable. At this temperature the primers can anneal to the single stranded DNA template strands. DNA polymerase was also stable at this temperature and can bind to the primers.

Extension / *Elongation*- The temperature was then raised slightly to Taq polymerase's ideal temperature (70-75oC). At this temperature Taq polymerase can synthesize and elongate the target DNA quickly and accurately.

Table: PCR Master Mix for HBB Primers

- Double distilled water (114.4 µl),
- dntp (16 µl),
- 10x PCR buffer (20 µl),
- Forward Primer (RDB1) (8 µl),
- Reverse Primer (PCO6) (8 µl),
- Taq polymerase (1.6 µl).

1. A sufficient PCR master mix was made up in the given order mentioned in the above table for the number of samples in an ice bath.
2. Master mix was vortexed briefly, then centrifuged briefly and short spined at 2000 rpm.
3. 8 µl of master mix was pipette into each PCR tube and 2µl of template DNA was added.
4. Prepared reaction tubes were placed in thermal cyclers in the following cycling conditions.

PCR Cycle conditions
PCR conditions for HBB primers
PCR Conditions

Initial Denaturation	95°C – 5.00 mins
Denaturation	94°C – 1.00 mins
Annealing	58°C – 0.45 secs
Extension	72°C – 2.30 mins
Final extension	72°C – 7.00 mins
Hold	4°C – α (for ever)

Go to 2 – 4 for 35 Cycles

After the reaction completes, 2µl of each PCR amplicon sample was electrophoresed to check the amplification. PCR products of more than 400bp were electrophoresed at 120V in 2 percent agarose gel. The PCR products were then visualized under UV light in trans illuminator. On obtaining a single band devoid of any primer-dimer bands the PCR products were sequenced.

Cycle sequencing conditions:

Sequencing Protocol

RR (Ready Reaction) Mix (Big dye)	- 1.0µl
Dilution Buffer	- 1.5µl
Primer (Forward or reverse)	- 2.0µl
PCR product	- 1.0µl
Milli Q	- 4.5µl
Total	- 10.0µl

Plate Processing: 5ml absolute alcohol was added to 200µl of 3 Molar sodium acetate (pH 5.2) in a tube. The tube was mixed thoroughly. 52 µl of the above mixture was added in each well of the plate. The plate was centrifuged at 4000 rpm for about 20 min in Eppendorf (5810R) centrifuge at 25ºC. The plate was then inverted to remove the supernatant. 100 µl of fresh 80% ethanol was added to each well and again centrifuged at 4000rpm for 20min. The plate was once again inverted and placing filter paper and giving a short spin for 10 seconds at 750rpm for removing ethanol.

The plate was covered properly with fresh foil. At the time of sequencing, 10µl of 50% HiDi™ formamide was added to all the wells. The sample plates were kept and run in the ABI Prism® 3730 DNA Analyzer (for sequencing).

DNA Analyzer: ABI PRWASM® 3730 DNA Analyzer- automatically analyses DNA molecules labelled with multiple fluorescent dyes. It consists of a charge couple device (CCD) camera and a power computer that includes software for data collection and data analyses. After samples are loaded onto the system's vertical gel, they undergo electrophoresis, laser detection, and computer analyses. Electrophoretic separation can be viewed on computer screen.

Sequencing

Molecular detection by sequencing was done on the ABI Prism® 3730 DNA Analyzer platform.

Beta globin gene reference sequence

The generated sequences were aligned to the β globin gene reference sequence with the use of Seq Scape v 2.5 software (Applied Bio-systems, USA). Seq Scape was one of the suits of Applied Biosystems designed for automated sequence data analyses. It performs sequence comparisons for variant identifications, SNP discovery and validation. It allows analyses of the re-sequenced data, comparing the consensus sequences to a known reference sequence. The reference sequences for the β globin gene were obtained from NCBI GenBank data base.

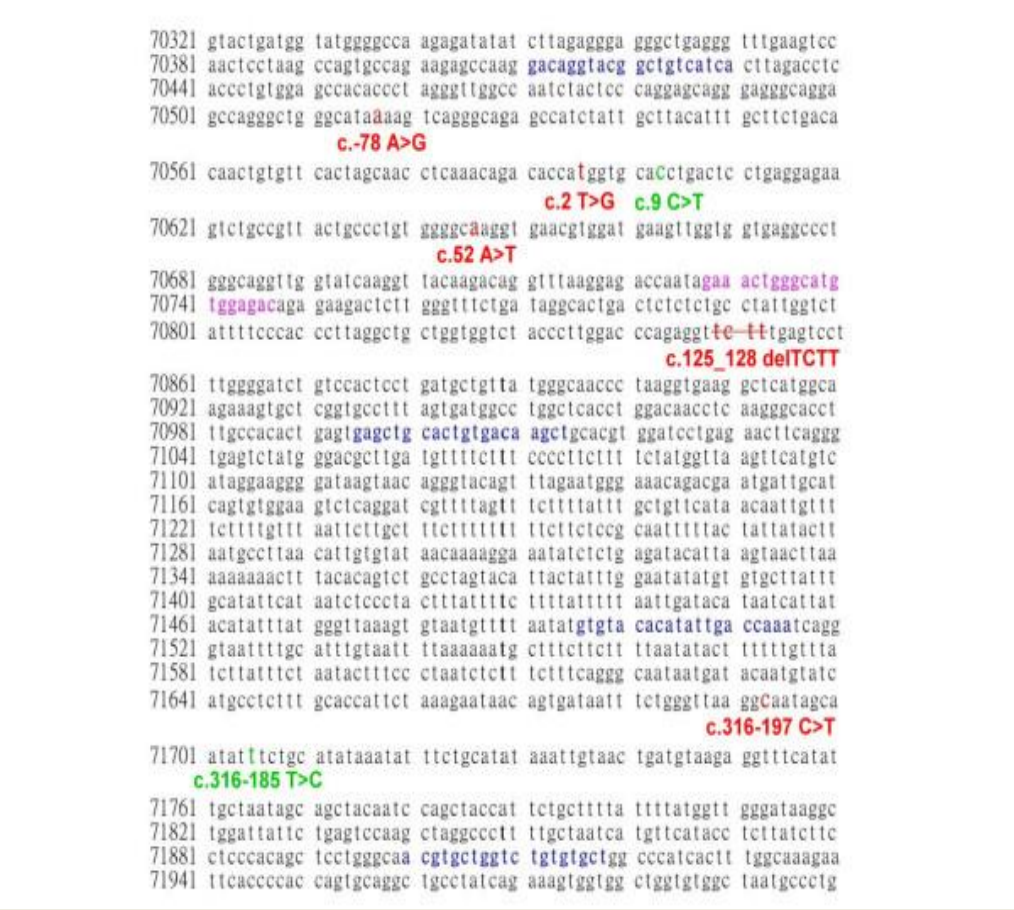


Figure 1.10: Beta globin gene sequence showing mutations and variation sites.

Statistical tools used

Validation of CBC cut off against Mean and SD for haematological parameters were done. Total individuals are divided into three parts based on complete blood count indicative marker value. Then the mean and standard deviation value of both sets, indicative and non-indicative was calculated. Also, the t-value (t-test) in comparison both set's indicative and non-indicative.

From all the laboratory tests, the statistical tests were performed to eliminate the normal samples and to confirm the β thalassaemia traits and other haemoglobin variants. At first stage all the data have been divided into three parts one was indicative, second was non-indicative and third was suspected based on different haematological parameters. The mean and SD value calculated of all the indices of RBC, the percentage value of HbA_2 and the percentage of HbE. Lastly, the p-value was calculated from different haematological parameters and haemoglobin fractions.

Area of Study

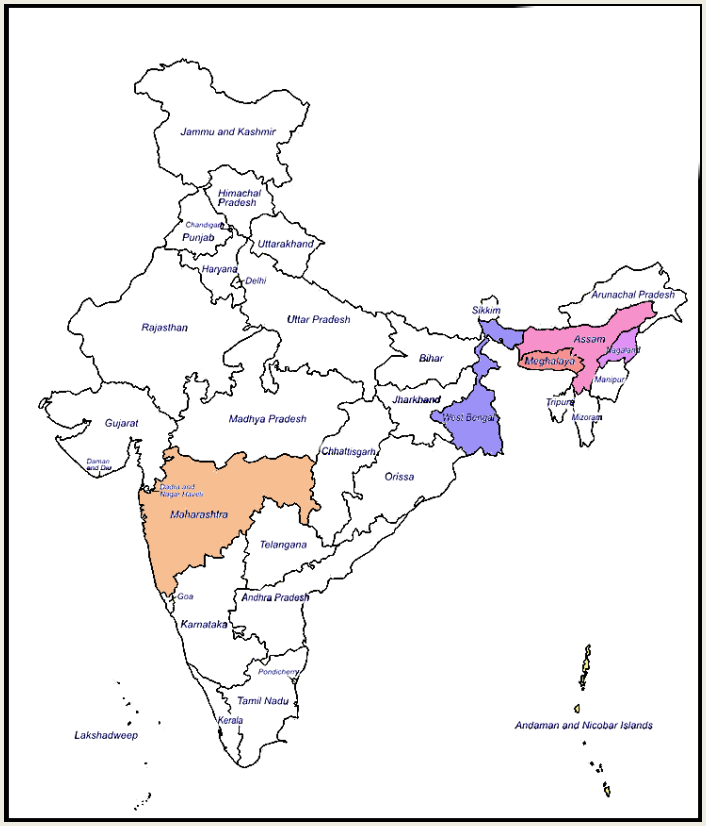


Figure 1.11: Map of India showing area of screening for haemoglobinopathy

Table 1.2: Total individuals screened for haemoglobinopathies from different regions in India

Region	No. of Samples Screened
Andaman and *Nicobar*	432
Central	6463
Eastern	8676
Northeastern	1839
Total	17410

The total number of individuals screened from Andaman and Nicobar Islands, Central region, Eastern and North-eastern regions are given in table 1. A total of 17410 individuals were screened under the project 'Community Genetics, Health and Bio-Cultural Diversity' by Anthropological Survey of India during the years 2005-2012.

Chapter 02

HAEMOGLOBINOPATHIES IN ANDAMAN AND NICOBAR ISLANDS

Figure 2.1: Map of Andaman and Nicobar Islands showing Port Blair

Andaman & Nicobar Islands had only been inhabited by the tribes, before colonial invasions in the seventeenth century and thereafter by various European agencies. Annexing of these Islands with colonial rule in the mid-nineteenth century by the British; drastic changes appeared in these Islands in terms of high magnitude of population influx there, as colonization policies. As soon as these Islands came under colonial power in 1858; the ten administrative headquarters at Port Blair and its surroundings were flooded by 15000 migrants (Man 1883) from various parts of the undivided Indian and neighbouring colonial establishment of Burma. Processes of colonization took its momentum with the establishment of befriending contacts with the native islanders throughout these Islands. Simultaneously, the gradual increase of the migrant population from diverse geographical and cultural backgrounds became phenomenal in the next decades, which was still in the process of framing aspects of the Islands' societies including population dynamics and diseases profile.

During the course of 150 years and above, except for a few, the rest of the native tribes have been marginalized in the fold of exotic culture and the Andaman & Nicobar Islands have emerged with a new identity, Mini-India-melting pot of Indian diasporas; where 'Local' (prisoners, convicts and their descendants, formed between 1885-1942), Coorgi (Kodagu district of Karnataka), Burmese (during 1907-1923), Moplah (in 1921 from Malabar coast), Karen (hunter-gatherer tribes from bordering districts of Myanmar and Thailand; during 1925-1927),

Bhatu (de-notified wandering tribe of bordering districts of United Province and Central Province, during 1926-1928), up-rooted Bengalese from East Pakistan (during 1949-1970), repatriated Tamil and Telugu of Burma (during 1950-1962), Ranchi-wala (tribal and non-tribal communities from Chhotanagpur region, arrived in 1950's). Several Malayalam families (during 1952-1958), and families of Soldiers, Sailors, Airmen Board of Ex-serviceman Association and Indian Ex-serviceman Association (belonging to Punjab, Kerala, Maharashtra, Tamil Nadu during 1969-1980 too have appeared as major ethnic components of colonized Islands in the 20th century (Singh 1994).

Interestingly, these migrant populations of present-day Andaman and Nicobar Islands are the counterparts of various caste-based endogamous groups of all streams of lingual groups. Moreover, their migration to these Islands had been guided by socio-political compulsion and not by choice. The search for luck and habitat was the primary reason for settling over decades in the island situation. Significantly, their places of origin in pre- and post-independent eras, belong to those specific geographic zones of India's mainland and neighbouring states which have already been identified as endemic for various types of abnormal haemoglobin genes among the natives of those areas.

- Apart from the nation-wide scattered distribution of α-Thalassaemia carriers' areas around the northern part are endemic for HbD (Punjab) where ancestors of 'Local Born', Bhatu, Valmik, Ex-service Men and other ethnic groups arrived.
- vast areas eastern India and adjacent areas are endemic for HbE; where from Bengalee, Burmese, Karen, Nicobar's and Shompen migrated in different points of time.
- almost entire central part and some southern areas of the country are endemic zones for HbS; where from Coorg, Ranchi-wala, Telugu and Tamil migrated.
- coastal plain of Malabar borrows genetic signature of HbO (Arab), because of marriage relations with Arabian traders, where from Moplah migrated.

Apart from family units, migrants comprised more men than women, particularly in most biologically potential age groups; this has consequently led to setting very flexible marriage rules among the migrants in due course and had formed a consolidated settlers' communities, heterogeneous by nature, without having rigid cultural boundaries, as are prevailing at their place of origin.

Thus, in practice members are identified under the social category of a 'lingual community', like Bengali, Telugu, Tamil and so on. However, that was identified and based on the mother tongue of the father of the individual. The extended family history of three to four generations depth often shows members of different ethnic identities. As members do not strictly follow the rule of endogamy (regarding language or caste) for marriage. Marriage with members of different linguistic communities was practiced. Two numerically prominent communities, 'Ranchi-wala' and 'Local', denote a large heterogeneous group, comprising the descendants of tribe/non-tribe ethnic groups of Ranchi (Chhotanagpur region) and the descendants of convicts respectively.

It has been established that migration has played an important role in our evolutionary process. The migrations consequently would have affected diseases susceptibility in some ethnic groups (Neel, 1969; Ward et. al., 1980; Young et. al., 1990; Weatherall 2000). Large-scale movements of certain ethnic backgrounds from ecological settings to other places and subsequently established mating relationships with local populations may lead to changes in diseases patterns. (Smouse and Teitelbaum, 1990; Rao et. al., 1992). Since, all new genetic variations are derived from mutation, genetic drift and random mating between species (individuals); large-scale human migration into these Islands might have played an important role in spreading genes and in the prevalence of fatal disorders like haemoglobinopathies in successive decades.

Details of health camps

The study, conducted during the years 2007-2006, primarily in the city of Port Blair, targeted students from various academic institutes. (Table 2.1). This study was however, extended to the individuals of Great Andamanese, Onge and Shompen; in response to the request of Andaman *Adim Janjati Vikas Samity*, and A & N Administration.

Table 2.1: Camp wise Screening Conducted in Port Blair and Abnormalities identified

Date of Camp	Place of Camp	No. of Samples	CBC Suspected Samples	Abnormalities						
				BTT	HbAE	HbAS	HbEβ	HbFH	BTT Major	HbSβ
12.12.2007	AAJVS,Port Blair	19	7		2		1			
13.12.2007	JNR&GB Pant Hospital	97	33	2	2	2				
25.1.2008	JNR College	29	10	3	1		1			
31.1.2008	HWI, Port Blair	24	3			1		1		
8.2.2008	JNR College	50	10	2	1	1				
12.2.2008	JNR College	38	3			1				
17.2.2008	JNR College	35	8	1	2	1				
24.2.2008	GBP Hospital	17	8	3	1		1	1		
29.2.2008	GBP Hospital	18	17	9	1	1			1	1
30.5.2008	GBP Hospital	13	8							
26.6.2008	GBP Hospital	37	5		1			1		
7.7.2008	JNR&GB Pant Hospital	25	10	1	1					
27.08.2008	GB Pant Hospital	30	13	1	2				2	
Total		432	135 (31.25)	22	14	7	3	3	3	1

A total of 432 unrelated students from various academic institutes of Port Blair town participated in the camps. Besides them, 19 members of Particularly Vulnerable Tribal Groups (PVTG) too participated in the study. All the samples were subjected to CBC out of which 135 (31.25 percent) were found suspected for haemoglobinopathies.[1] Distribution of screened individuals by language identity was given in Table 2.2.

[1] In Andaman, NESTROFT solution could not be preserved and used in field because of the high temperature.

Table 2.2: Distribution of screened individuals by language identity.

Language identities	Academic Institutes					PVTG (N:19)	Total (N:432)
	I (N:51)	II (N:91)	III (N:199)	IV (N:24)	IV (N:48)		
Bengali	22	27	70	07	37		163
Ranchi-wala	04	11	55	01	03		74
Tamil	00	11	22	02	04		39
Telugu	13	17	05	03	02		40
Malayalam	00	08	10	02	00		20
Kannadi	06	00	00	00	00		06
Local Born	04	10	00	02	00		16
Nicobarese	02	01	25	00	00		28
Burmese	00	00	01	00	00		01
Punjabi	00	01	02	02	00		05
UP	00	04	08	01	01		14
Bihar	00	01	00	00	00		01
Karen	00	00	00	01	00		01
Nepali	00	00	01	00	01		02
Bhatu	00	00	00	03	00		03
Great Andamanese						16	16
Onge						02	02
Shompen						01	01

Results and discussion:

Haematological parameters

This study was carried out among 432 unrelated individuals of both genders, having various ethnic backgrounds, including 19 individuals from Particularly Vulnerable Tribal Groups of these Islands.

Table 2.3: Mean and SD of Haematological parameters of Suspected and Normal Cases.

Haematological parameter	Haemoglobinopathies (n=53)		Normal n= (379)	
	Mean	SD	Mean	SD
RBC (*106/µl)	4.218	0.6596	4.42	0.5046
Hb(gm/dl)	8.44	2.665	11.65	1.873
Hct (percent)	28.06	7.437	36.41	5.377
MCV (fl)	65.62	9.919	82.31	3.801
MCH (pg)	19.58	4.452	26.18	1.576
MCHC (gm/dl)	29.64	2.592	31.91	0.702
RDW	11.32	2.61	9.766	0.542

Table 2.3 gives the mean and standard deviation of the suspected cases as observed from CBC (haematological) parameters. The figures below the cutoff values were considered suspected and the means values of all the parameters are lower than the normal cases.

Out of the total 432 participants, 53 individuals had been detected under various categories of haemoglobinopathies at various stages of severity. β-Thalassaemia Major was detected with a frequency of 0.694 percent and the carrier of the same disorder was estimated to be moderately high (4.86 percent), whereas HbE and HbS carrier frequency was 3.24 percent and 1.62 percent, respectively. Significantly, three individuals with compound heterozygote for HbE / B-Thalassaemia and one individual for HbS/β-Thalassemia were detected respectively (table 2.4). Significantly, the detection of one individual of Delta-B Thalassaemia with the frequency of fatal haemoglobin (14 percent) and high A_2 with apparently normal haemoglobin levels (13.3 gm/dL.), RBC (5.91ˆ106/µl.) count but significantly low MCV (68.8 fl.) and MCH (22.5 pg.), was considered a rare abnormality.

Table 2.4: Prevalence of haemoglobinopathies among screened individuals

Haemoglobin Variants	Results of Different Camps					PVTG (N:19)	Total (N:432)
	I (N:51)	II (N:91)	III (N:199)	IV (N:24)	IV (N:48)		
B-Thal Major	2	1	0	0	0	0	03 (0.694 percent)
B-Thal Trait	8	8	3	0	2	0	21 (4.86 percent)
Hb AE	6	1	3	0	2	2	14 (3.24 percent)
Hb AE/Tt	2	1	0	0	0	0	03 (0.694 percent)
Hb AS	2	0	4	1	0	0	07 (1.62 percent)
Hb AS/Tt	1	0	0	0	0	0	01 (0.23 percent)
HbA/AE	0	0	0	0	0	0	0 (0.0 percent)
HPFH	3	0	0	0	0	0	03 (0.694 percent)
Delta-B-Thal	1	0	0	0	0	0	01 (0.23 percent)
Total	25	11	10	1	4	2	53 (12.26 percent)

Molecular characterization of the 53 BTT samples revealed that two β-Thalassaemia carriers/traits had common IVS-1-5-G>C mutation. One of those also co-inherited alpha Thalassaemia 1 with more severity. One case of HbE carrier confirmed Cd 26 G>A mutation. Significantly one case of HbE-β-Thalassaemia confirmed IVS-1-5-G>C and Cd 26 G>A mutation, which was also the case of a co-inherited Alpha Thalassaemia 1 with devastating clinical outcome.

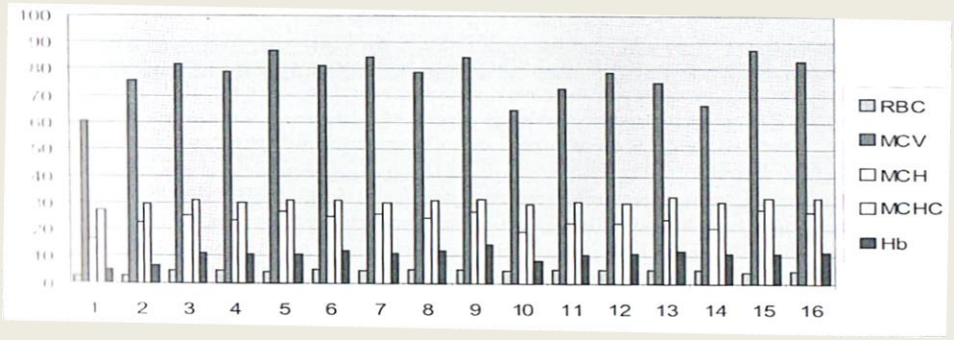


Figure 2.2: Individual variation in RBC, haemoglobin and RBC indices in Great Andamanese samples.

Out of 19 Great Andamanese, two were found to be HbE carriers with 31.1percent and 29.9 percent A_2 respective to those individuals, who though had RBC count of apparent normal range i.e. 4.72ˆ106/µl. and 5.01ˆ106/µl, respectively. Apart from that, a general trend of anaemic state has also been observed among Great Andamanese individuals, who have participated in this study. Ranges of their red blood cell count (RBC), haemoglobin level and red blood cell indices are shown (Figure. 2.2) for better understanding of their haematological health.

Implication of prevalence of haemoglobinopathies for members of island communities

Important demographic feature of Andaman & and Nicobar Islands has been a remarkable imbalance in the sex ratio. The higher rates of male migrants resulted in a low sex ratio. This also resulted in marriage across the ethnic groups formed largely based on the languages spoken by them. Such marriages of course lead to socio-cultural cohesion among the islanders and further increase of intercommunity marriages.

Inter-ethnic marriages possibly provide the opportunity for increased cases of combined heterozygosity like HbAE/BTT, and HbAS/BTT. Simultaneously, the maintenance of traditional marriage rules in island scenarios within a low sex ratio keeps probabilities more open for increasing haemoglobinopathies in homozygote as well as heterozygote conditions in coming generations. A few case studies presented below illustrate the above describes circumstances better.

Case-I: A girl of 5th standard, was a patient of B-thalassaemia Major. She was detected at the age of 10 years. She belonged to a ‘Local' born settler family, whose grand grandparents were the natives of diverse eco-cultural zones. She was the second daughter of her parents. Her parents were only concerned about her prolonged episodes of illness and blood transfusion at regular intervals. That little girl could survive hardly a few months after her case was properly diagnosed by the project team of the Anthropological Survey of India. Later, it was decided to detect the opportunity of diseases in some of the members of her family and close kin. This study found that both of her parents carried B-thalassaemia traits as also her elder sister.

Those findings further encouraged investigating on extended genealogies of that family. Finally, some of the members of close kin groups voluntarily approached the study team for the detection of the disorders. This led to detection of some cases of carrier genes for B-thalassaemia, Hb E and one combined case of B Thalassaemia trait and Hb E (β-Thal/HbAE).

Case-II: It was a case of a girl of 18 years, who was detected B-thalassaemia carrier/trait by the study team. She revealed that her younger brother had been going through regular blood transfusions and had splenectomy in the local hospital. She was then requested to meet with her parents and discuss the facts. Finally, both of her parents and relatives of paternal and maternal line came forward voluntarily for clinical detection. Simultaneously an extended genealogy was also obtained based on the diagnosis of their' samples. It appeared that families originally belonged to some district of the south-central part of India, and they have migrated three decades ago in search of better livelihood opportunities in these Islands. Since their arrival to these Islands was comparatively recent, they are adhering to their native culture and strictly followed preferential rules of marriage in the island's scenario. It was found that consanguineous marriages between cousins and consanguinity up to second-degree relatives, ultimately saw to that

abnormal haemoglobin genes were retained within the family and relatives. It appeared that where cross- cousin marriages were strictly adhered to B-thalassaemia major and/or B-thalassaemia carrier/trait cases had been naturally eliminated from members of next-generation.

Case-III: It was a case of a Great Andamanese boy of 12 years, who was detected as a carrier of HbE (HbAE) having normal haemoglobin (11.2 gm/dL) level and normal RBC count (5.01^106/Al.) but with very high frequency of A_2 (29.9percent) through HPLC column detection. It was observed that both of his parents belong to the Great Andamanese tribe, who had genetically never carried any type of mutant haemoglobin genes, as they remained as breeding isolated human groups of the Andaman alike Jarawa and Onge. However, the fast transformation of these Islands under rule; had hardly left any option for them to maintain a traditional way of life. Marginalization, and rampant depopulation since the late mid-nineteenth century; compelled the Great Andamanese to be assimilated with non-traditional systems of survival; including selection of mates from migrant communities like Karen, Burmese, Ranchi-wala during the initial decades of the 20th century and from Bhatu, Moplah, Bengalee during the last few decades.

Marriage alliance with a Burmese person of that boy's mother line has been identified through an extended genealogy, which could have been the root of acquiring HbE in the family. However, both of the parents of that boy were not available during the screening programme, which could throw light on understanding the flow of HbE; as one of the elder sister of his father was also detected as a carrier of HbE.

The dwindling population size with a low sex ratio (overall 88 females over 100 male members among only 55 population strength in 2007) of the Great Andamanese inevitably put them on the threshold of acquiring many genetically controlled diseases/disorders, including haemoglobinopathies through random selection of marriage partner from non-Great Andamanese settler communities.

Discussion

The scenario of haemoglobinopathies in the public health domain of Andaman and Nicobar Islands was no exception. The high prevalence rate alone in B-Thalassaemia carrier/trait i.e. 4.86 percent (higher than the national average, which was 3.96 percent), endorse the threats in terms of increasing a load of carriers and patients in these Islands due to the nature of demographic and social dynamics.

Noteworthy to mention that before blood sample collection; the study team initiated an awareness programme on some pertinent issues of haemoglobinopathies among the students. Surprisingly, the students had little knowledge even on the very basics, like the cause of the diseases, affected organ/body part of the diseases, consequences of the diseases and transmission. The awareness programme was then initiated from the preliminary level of haemoglobinopathies. The study threw light on enigmas. There was no information on the detection of such large-scale screening of haemoglobinopathies earlier in these Islands. We had to walk miles not only for preventive and clinical management of the disorders but also to understand the silent contribution of haemoglobinopathies to infant and child mortality rates from the public health point of view.

Awareness address to school children for sickle cell anaemia and thalassemia

Solubility/NESTROFT test and blood collection during field operation.

Chapter 03

HAEMOGLOBINOPATHIES IN CENTRAL REGION

In the central region, screening was conducted in the state of Maharashtra in the Vidarbh region. Vidarbh region was situated in eastern part of the state of Maharashtra, and borders the state of Madhya Pradesh to the north, Chhattisgarh to the east, Telangana to the south and Uttar Maharashtra region of Maharashtra to the west.

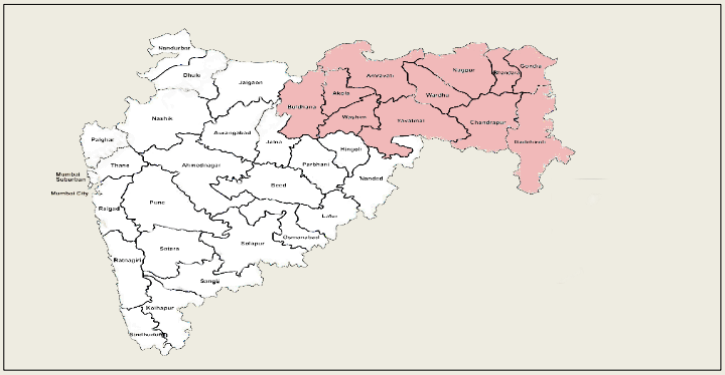


Figure 3.1: Map showing the regions of screening in Maharashtra.

The total number of individuals screened from the Vidarbh region of the state of Maharashtra are 6463, comprising of 3192 males and 2755 females. Four districts were covered as given in Table 3.1.

Table 3.1: District wise screening in Vidarbh Region.

District	Total No. of Samples
Nagpur	2432
Chandrapur	155
Gondia	495
Yeotmal	3381
Total	6463

Table 3.2: Details of camps and number of individuals screened.

S.No	Year	Place of Camp	No. of Samples
1.	2005	S.M. Jaibai Choudhari Gyanpeeth School, Sadar, Nagpur	166
2	2005	Yugantar High School, Sadar, Nagpur	151
3	2005	St. Joseph Vidyalaya, Nagpur	107
4	2006	Shri. Krwashna Madhyamik Vidyalaya, Nagpur	64
5.	2006	Jawahar Navodaya Vidyalaya, Chandrapur	437
6	2006	An.S.I.CRC Nagpur	6
7	2006	Shantiviya Bhavan, Mankhija Junior College, Nagpur	421
8	2007	Jawahar Navodaya Vidyalaya, Gondia	495
9	2007	Priyadarshini Hall, Chandrapur	155
10	2007	Raman Science Centre, Nagpur	490
11	2006	Sindhi Hindi High School, Nagpur	590
12	2008	Yeotmal	3381
		Total	6463

Screening by solubility test

The initial screening was done by the solubility test. The samples positive for sickle cell was brought to the laboratory of Central Regional Center, Nagpur for further analysis and confirmation.[2]

Haematological Parameters:

Screening was conducted among different communities in the region. A total of 6463 individuals was screened.

Table 3.3 gives the red cell morphology for HbS and HbβT. From the table, it was observed that carrier HbS pooled data showed higher mean values of WBC, HGB, MCV, MCH, MCHC and RDW but lower values of RBC and PLT higher than those for HbβT.

The red cell count was relatively higher in relation to the haemoglobin, MCH and MCHC in the HbβT carrier than that found in the HbS carrier, the situation leads to a common feature of iron deficiency in HbβT carrier individuals. Lower values of WBC and RDW accompanied by higher RBC in β- thalassaemia carriers encountered with hypochromic and microcytic red cell indices.

Table 3.3: Red cell morphology among carriers of HbS and HbβT individuals

HbVariant	Sex	No	Mean	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT
HbAS	M	115	M	9.1	6.5	12.4	48.7	75.5	20.7	27.75	14.1	417.3
			SD	3.1	2.3	2.3	16.4	14.9	6.1	9.0	2.6	284.2
	F	78	M	9.4	5.9	11.8	45.2	78.1	23.4	28.4	15.0	385.3
			SD	3.2	2.0	1.9	13.9	14.9	13.8	10.5	2.7	162.8
$Hb\beta^T$	M	52	M	8.6	8.1	12.1	50.6	63.8	15.8	25.3	12.9	457.4
			SD	3.0	2.9	2.2	17.4	11.3	3.7	4.9	1.7	246.4
	F	58	M	8.4	7.1	10.8	43.8	61.7	15.9	25.9	12.6	446.5
			SD	2.9	2.2	1.8	14.1	8.9	3.6	5.7	1.9	208.7

The red cell count was relatively higher in relation to the haemoglobin (microcytosis), MCH and MCHC in the HbβT carrier than what is observed in the haematological parameters of HbS carrier. This situation leads to a common characteristics of iron deficiency in HbβT carrier individuals. Lower values of WBC and RDW accompanied by higher RBC in β- thalassaemia carriers is encountered with hypochromic and microcytic red cell indices.

[2] Data on how many were tested positive for the solubility test was not available for Central region.

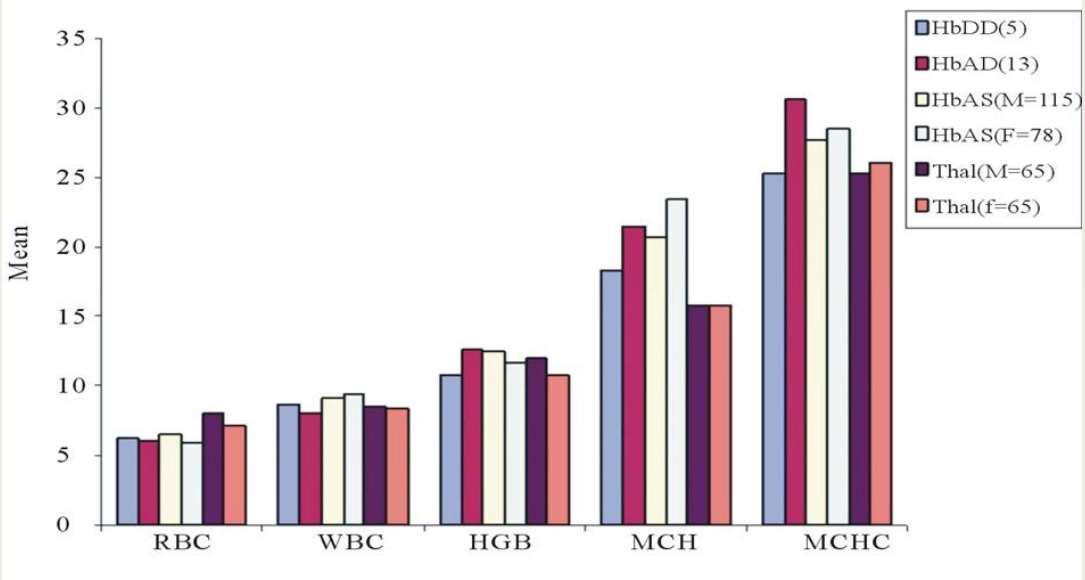


Figure 3.2: Distribution of Mean of cell morphology among different haemoglobinopathies

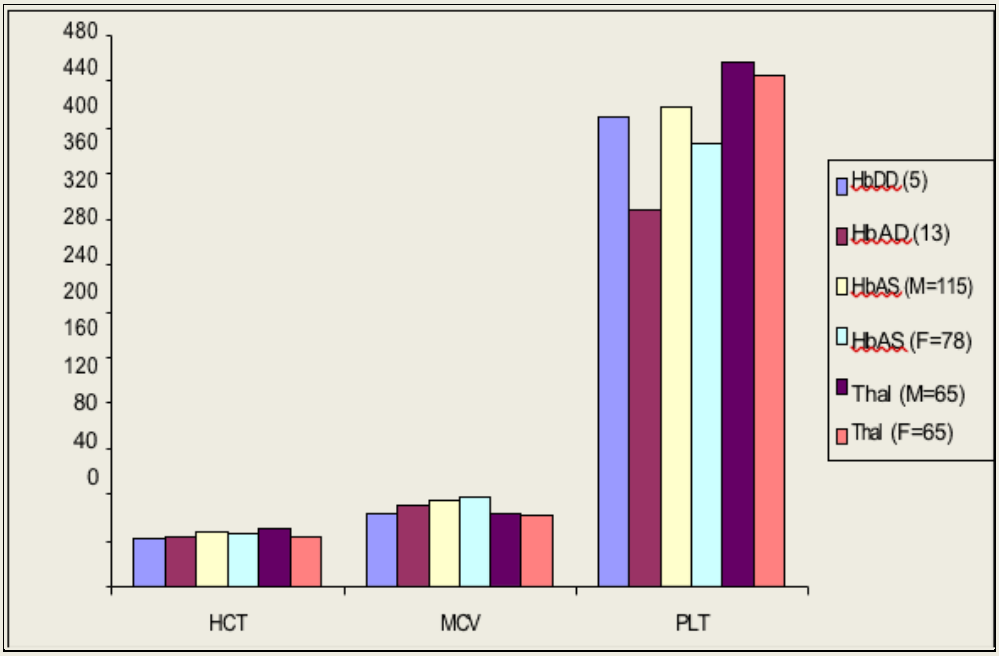


Figure 3.3: Distribution of Mean of Cell Morphology

Capillary electrophoresis

The suspected samples were subjected to capillary electrophoresis to confirm the percentage of haemoglobinopathies in each of the communities. Capillary electrophoresis differentiated the individuals into AA, AS, SS, SF, AS-A_2, A_2, A_2F.

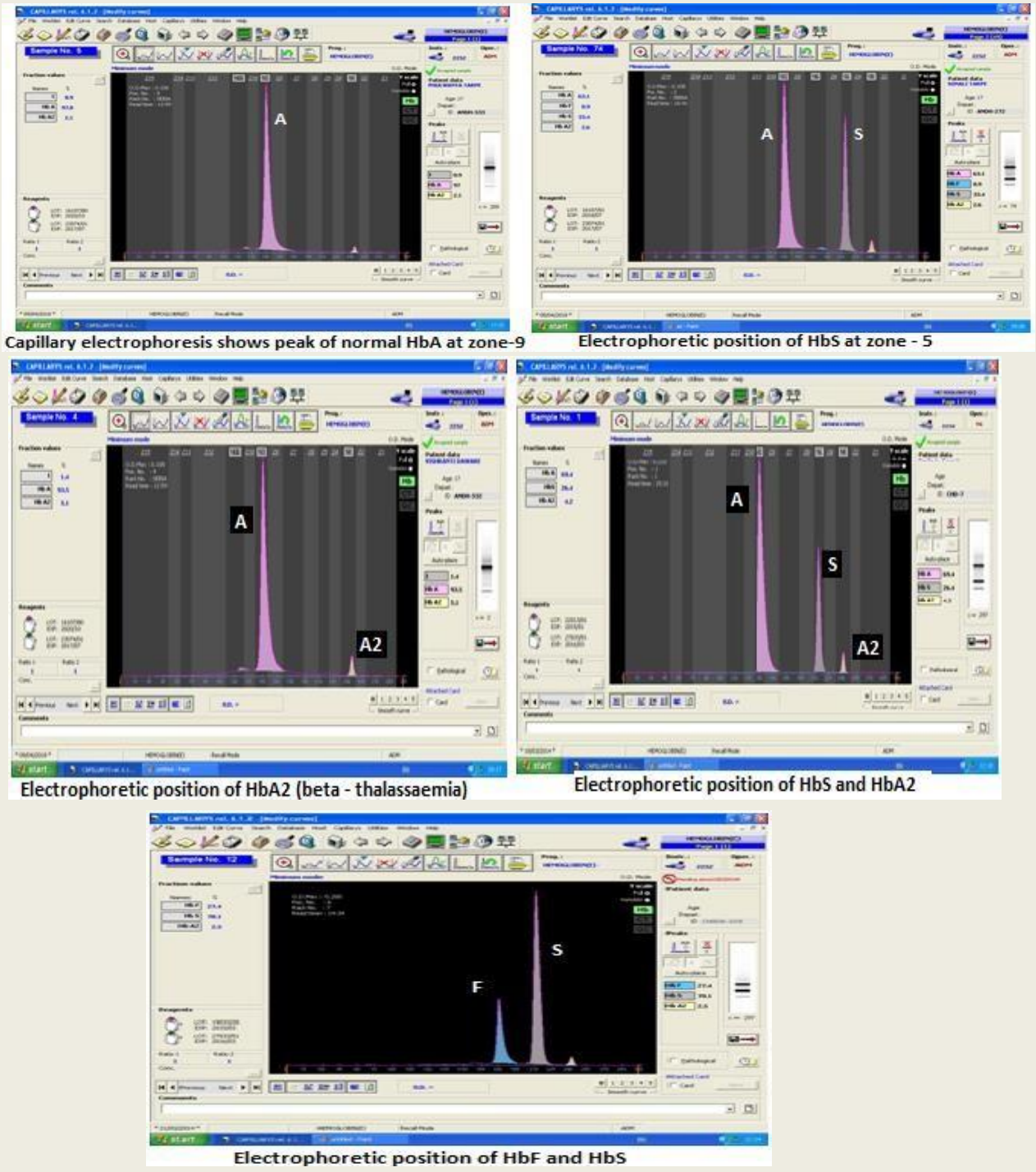


Figure 3.4: Capillary electrophoresis showing peaks of different Hb- variants

Table 3.4: Frequency distribution of different haemoglobin variants

Communities	No.	HbAA		HbAS		HbSS		β-thal		HbAD		HbDD	HbF
		M	F	No.	percent	No.	percent	No.	percent	No.	percent	No.	No.
Mahar	1651	751	699	195	11.81	8	0.48	-	-	-	-	-	4
Kunbi	185	77	99	9	4.86	-	-	-	-	-	-	-	-
Teli	329	177	140	12	3.65	-	-	-	-	-	-	-	-
Halba	139	86	49	2	1.44	-	-	1	0.72	1	0.72	-	-
Gond	230	103	99	27	11.74	-	-	1	0.43	-	-	-	-

Gowari	55	29	22	4	7.27	-	-	-	-	-	-	-	-
Marar/Mali	118	40	59	19	16.10	-	-	-	-	-	-	-	-
Bawane kunbi	23	11	11	1	4.35	-	-	-	-	-	-	-	-
Maratha kunbi	48	17	30	1	2.08	-	-	-	-	-	-	-	-
Dange kunbi	18	8	9	1	5.56	-	-	-	-	-	-	-	-
Kalar	111	56	49	6	5.41	-	-	-	-	-	-	-	-
Brahmin	144	89	49	6	4.17	-	-	-	-	-	-	-	-
Tirale kunbi	134	75	52	6	4.48	-	-	1	0.75	-	-	-	-
Khaire kunbi	246	94	129	23	9.35	-	-	-	-	-	-	-	-
Zade kunbi	13	7	5	1	7.69	-	-	-	-	-	-	-	-
Chambhar	54	28	25	1	1.85	-	-	-	-	-	-	-	-
Dhangar	43	25	17	1	2.33	-	-	1	2.33	-	-	-	1
Dhiwar	55	24	26	5	9.09	-	-	-	-	-	-	-	-
Pardhan	64	22	28	13	20.31	1	1.56	-	-	-	-	-	1
Kohali	37	16	20	1	2.70	-	-	-	-	-	-	-	-
Bania	11	8	2	1	9.09	-	-	-	-	-	-	-	-
Banjara	17	10	4	1	5.88	1	5.88	1	5.88	-	-	-	-
Muslim	161	117	38	6	3.73	-	-	-	-	-	-	-	-
Mehetar	62	29	32	1	1.61	-	-	-	-	-	-	-	-
Madgi	40	21	15	4	10.0	-	-	-	-	-	-	-	-
Dhobi	43	24	15	2	4.65	-	-	2	4.65	-	-	-	-
Rajput	49	28	18	1	2.04	-	-	1	2.04	-	-	1	-
Powar	113	57	52	2	1.77	-	-	4	3.54	-	-	-	2
Sutar	30	14	16	-	-	-	-	-	-	-	-	-	-
Lohar	59	26	32	1	1.69	-	-	-	-	-	-	-	-
Dhanoje kunbi	2	-	2	-	-	-	-	-	-	-	-	-	-
Lewa kunbi	4	1	3	-	-	-	-	-	-	-	-	-	-
Lonare kunbi	3	2	1	-	-	-	-	-	-	-	-	-	-
Katia	2	-	1	1	50.0	-	-	-	-	-	-	-	-
Navi	17	12	5	-	-	-	-	-	-	-	-	-	-
Sikh	40	25	11	-	-	-	-	1	2.5	3	7.5	-	-
Kumbhar	16	7	9	-	-	-	-	-	-	-	-	-	-
Beldar	21	13	8	-	-	-	-	-	-	-	-	-	-
Bengali	12	9	3	-	-	-	-	-	-	-	-	-	-
Marwadi	12	10	2	-	-	-	-	-	-	-	-	-	-
Sindhi	1241	549	562	2	0.16	-	-	115	9.27	9	0.73	4	-
Shimpi	12	8	3	-	8.33	-	-	-	-	-	-	-	-
Telugu	21	15	1	4.8	-	-	-	-	-	-	-	-	-
Matang	3	1	2	-	-	-	-	-	-	-	-	-	-
Pardeshi	4	1	2	1	25.0	-	-	-	-	-	-	-	-
Chrwastan	28	22	6	-	-	-	-	-	-	-	-	-	-
Bawas	6	2	2	2	33.33	-	-	-	-	-	-	-	-
Others	607	386	209	-	-	-	-	-	-	-	-	-	-
Total	6463	3192	2755	362	5.6	10	-	128	1.98	13	0.21.0	5	8

Table 3.4 gives the frequency distribution of different haemoglobin variants viz., HbA, HbS, HbSS and HbβT. It was observed that 362 individuals (5.6 percent) were positive for HbS and 128 (1.98 percent) for HbβT. The frequency for HbS and HbβT varied between 0 and 33 percent and 0 and 10 percent, respectively, among all studied populations. A very high frequency of HbS was encountered among the Bawas (33.3 percent) the Pardeshi (25 percent), the Pardhan (23.8 percent) and the Marar (20.4percent). A moderate HbS frequency was observed among Dhiwar (12.8 percent), Gond (12.4 percent), Shimpi (11.1 percent), Mahar (10.9 percent), Madgi (10.0 percent), Khairekunbi (9.3 percent), Bania (9.1 percent) the Zadekunbi (7.7 percent), and the Gowari (7.3 percent). Significant frequencies of HbS were also observed among the Banjara (5.9 percent), the Dangekunbi (5.6 percent), the Kunbi (4.9 percent), the Telugu (4.8 percent), the Kalar (4.7 percent), the Bawanekunbi (4.3 percent), the Brahmin (4.2 percent), the Muslim (3.7 percent), the Tiralekunbi (3.6 percent) and the Teli (3.2 percent). Figure 3.4 gives the incidence of sickle cell anaemia and thalassaemia in Vidarbh region

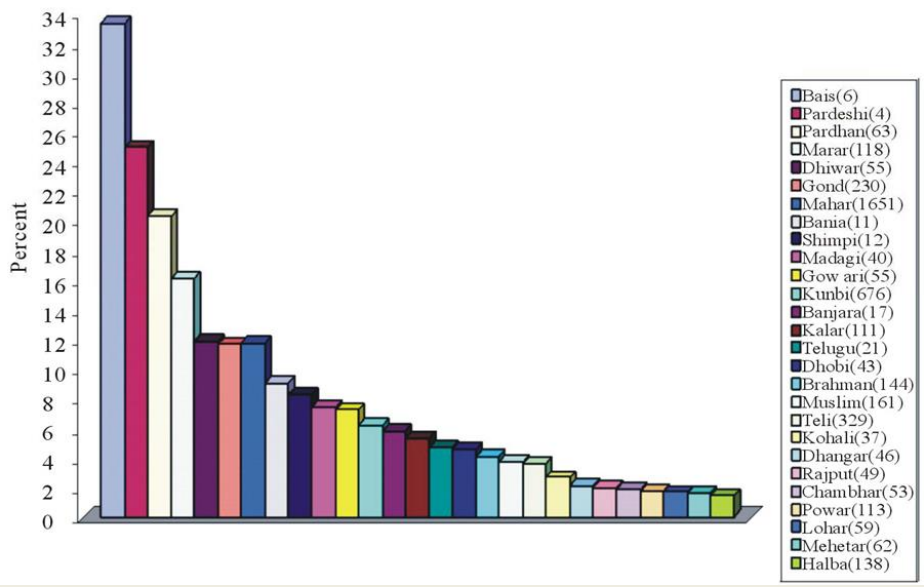


Figure 3.5: Incidence of Sickle cell anaemia and thalssaemia in Vidarbh region

The range of HbS gene frequency of 1-3 percent was accounted for the Chambhar, the Dhangar, the Dhobi, the Halba, the Kohali, the Lohar, the Maratha Kunbi, the Mehetar, the Powar and the Rajput of eastern region of Maharashtra. The number of homozygous HbS individuals were very few. The results also showed a moderate frequency of β-thalassaemia (9.27%) among the Sindhi. Few cases of β-thalassaemia in other tribal and caste groups were noticed. However, in some of the sub-groups and castes no abnormality of any Hb variant was detected.

The results show that the sickle cell trait (6.2 percent) was the most common type of haemoglobinopathies in the studied populations followed by β-thalassaemia carrier status (2.1 percent) and sickle cell diseases (0.2 percent).
Earlier studies showed a complete absence of the HbS gene in certain caste and religious groups like Brahmin (Sanghvi et al., 1962), Muslim (Hakim et al., 1972, Vijaykumar et al., 1987) and Dhangar (Undevia et al., 1985).

However, this study shows that the incidences of sickle cell gene vary from 2-4 percent even in these communities. Community-wise frequency distribution of β-thalassaemia in the region was given in figure 3.5.

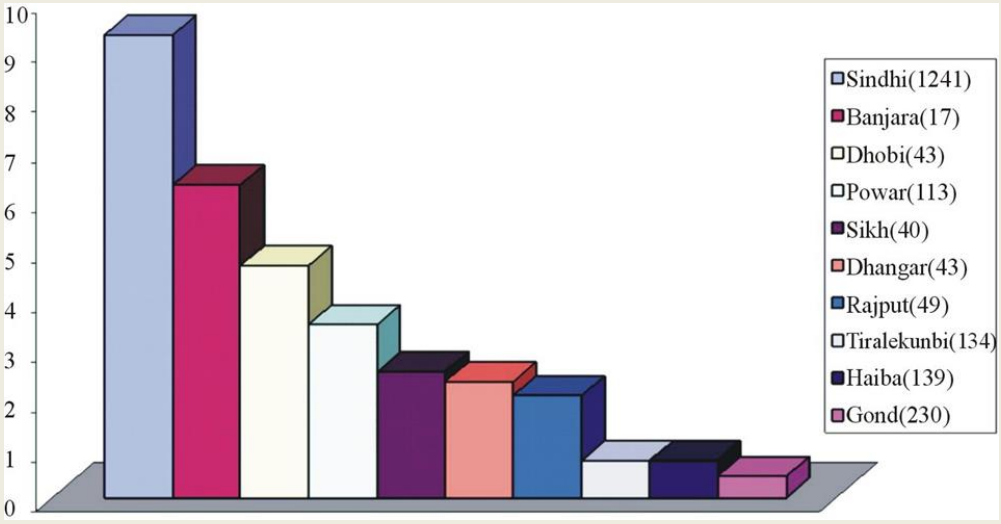


Figure 3.6: Community-wise frequency distribution of β-thalassaemia in Vidarbh region.

This finding was in conformity with earlier studies (Balgir, 2006, Sinha et.al, 2004). Further, sickle cell genes were noticed in Rajput and Kalar also with a range of 2-5 percent. The high frequency of sickle cell genes among the Pardhan was reported in earlier studies (Bhasin et.al, 1994, Brittenham, 1988) and observed in the present study. Earlier studies among the Mahar of central India, reported the frequency of HbS between 0 to 24 percent and that of sickle cell diseases between 0 to 6 percent (Urade, 2012, Das et.al, 1961, Mukherjee and Das, 1990, Kate, 2001, Negi, 1976, Lele et.al, 1962, Solanki et.al, 1967, Shukla and Solanki, 1958). The present findings show a moderate frequency of HbS among the Mahar and the Kunbi, thus differing with the results of earlier scholars who reported a very high frequency of HbS among these two endogamous groups which may be due to technical differences.

Molecular Characterisation

Total 78 sequences were assembled in respect of the reference sequences corresponding to GRCH37: 11: 5246697: 5248301: 1 covering Beta-gene including the 4 exons and the intervening introns. The following mutations were noted from the assembled segment:

1. 409t position corresponding to 5247105 of the reference sequence. 3 cases in heterozygous form of C>T have been observed at site 5247105 of the reference sequence. This mutation was not mentioned in the dbSNP.

2. 625 positions corresponding to 5247321 of the reference sequence. 2 cases in heterozygous form of A>T have been observed at site 5247321 of the reference sequence. This mutation was not mentioned in the dbSNP.

3. 922 positions corresponding to 5247618 of the reference sequence. One case in heterozygous form of A>G has been observed at site 5247618 of the reference sequence. This mutation was not mentioned in the dbSNP.

4. 962" position corresponding to 5247658 of the reference sequence. 1 case in heterozygous form of C>G has been observed at site 5247658 of the reference sequence. This mutation was not mentioned in the dbSNP.

5. 1096* position corresponding to 5247792 of the reference sequence. 3 cases in heterozygous form of C>G have been observed at site 5247792 of the reference sequence. This mutation was mentioned in the dbSNP.

6. 1458% position corresponding to 5248154 of the reference sequence. 4 cases in heterozygous form of C>G have been observed at site 5248154 of the reference sequence. This mutation was mentioned in the dbSNP.

7. 14627 position corresponding to 5248158 of the reference sequence. 1 case in heterozygous form of C>A has been observed at site 5248158 of the reference sequence. This mutation was not mentioned in the dbSNP.

8. 1535t position corresponding to 5248231 of the reference sequence. 5 cases in heterozygous form of T>A have been observed at site 5248231 of the reference sequence. This mutation was mentioned in the dbSNP.

9. 1546% position corresponding to 5248242 of the reference sequence. 29 cases in heterozygous form of A>G have been observed at site 5248242 of the reference sequence. This mutation was mentioned in the dbSNP.

The normal and mutated HBB sequence for HbS was as given below.

HBB Sequence in Normal m Adult Haemoglobin (HbAA):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys Ser

HBB Sequence in Mutant Adult Haemoglobin (HbAS):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG TCT
Amino Acid	Leu	Thr	Pro	VaI	Glu	Lys Ser

The present study observed the presence of HbS also among the higher caste communities. Occurrence of abnormal gene HbS and HbBB among the people of different communities was due to mutation in the genetic material (DNA). Sickle cell anaemia and thalassaemia are the best examples of point mutation as these disorders are caused by a single gene which was transmitted through parents to offspring from generation to generations. And this mutation has taken place independently in different communities in different areas across the world during human evolution.

The present study the presence of HbS gene in almost all castes and communities signifies that sickle cell anaemia was not confined to specific ethnic groups; instead, it was widely distributed in all tribal, scheduled caste, backward and higher caste populations' native to this region. Similarly, sporadic occurrence of HbBTT, HbD in the present study suggests the spread of HbBTT well beyond the Sindh and Punjab regions to central India. The high magnitude of HbS and BTT appears to contribute significantly to the load of haemoglobinopathies in this region which ultimately was a great challenge balancing the genetic constitution and threat to these populations in this region, since many of the sicklers survive till middle age.

Chapter 04

HAEMOGLOBINOPTHIES IN EASTERN INDIA

Screening in the eastern region was conducted in the state of West Bengal which has a rich history of culture and language. Situated in the eastern part of India, West Bengal has the Bay of Bengal to its south, and Bangladesh to the east. West Bengal was once the cultural nucleus of India with about 200 ethnic groups including OBCs, SCs, STs, PVTGs and 97 other communities. It claimed a very important political position during the British period. The northern part of West Bengal has a unique geo-ethnic characterization. The cultural specificities of the different communities and ethnic groups are of interest to the study of human variation. The state has a population of 91,347,736 (Census 2011) distributed in 23 districts.

The river Ganga has been one of the most important rivers in this part of the country influencing the occupations and food habits and ensuing age-old history of the settlements. Moreover, it was noted that owing to its one side-open to the sea geography, fertile land and access to further east, West Bengal has claimed the attention of many visitors for years. Dutch, Portugal, British and many other European invaders have established their colonies for over 700 years, whose traces are persisting in West Bengal. It was understood that in many of these colonies the local inhabitants, caste people and indigenous ethnic groups were used as labours and bonded servants, which led to a forceful revelation to the elements of outsiders. Instances of religious conversions are still prominent. These facts and events have gradually paved the way for admixture. Left out signature of the genetic materials of the colonizing Europeans in this part of the country was not rare. Until recently events of migration and settlement of people from Bangladesh have been noted in West Bengal.

All these historical and recent episodes of possible admixture make this area sensitive for the study of genetic disorders. Recent studies undertaken on haemoglobinopathies in West Bengal have reported a higher frequency of β thalassaemia (Bandopadhyay et al 1999), and structural variant haemoglobin (Das et al 2000, Das et al 1991, Deka et al 1988). However, less was known about the ethnic diversity of the haemoglobinopathies of people of West Bengal, as well as the distribution of mutations among them. The present project was initiated in this part of the country to address these issues. However, the project also aimed at generating public awareness regarding haemoglobinopathies in Eastern India.

Area of Study

In West Bengal, the area chosen (Figure 4.1) for the present study initially was southern-most point of South 24 Parganas, covering two blocks. Screening was carried out in the districts of East and West Midnapur, North and South 24 Parganas, Howrah, Hooghly and Murshidabad. A cross-sectional survey was performed to assess the level of awareness among the residents of the areas. Care was taken to include only the individuals who have a residence history of more than three generation in the study location.

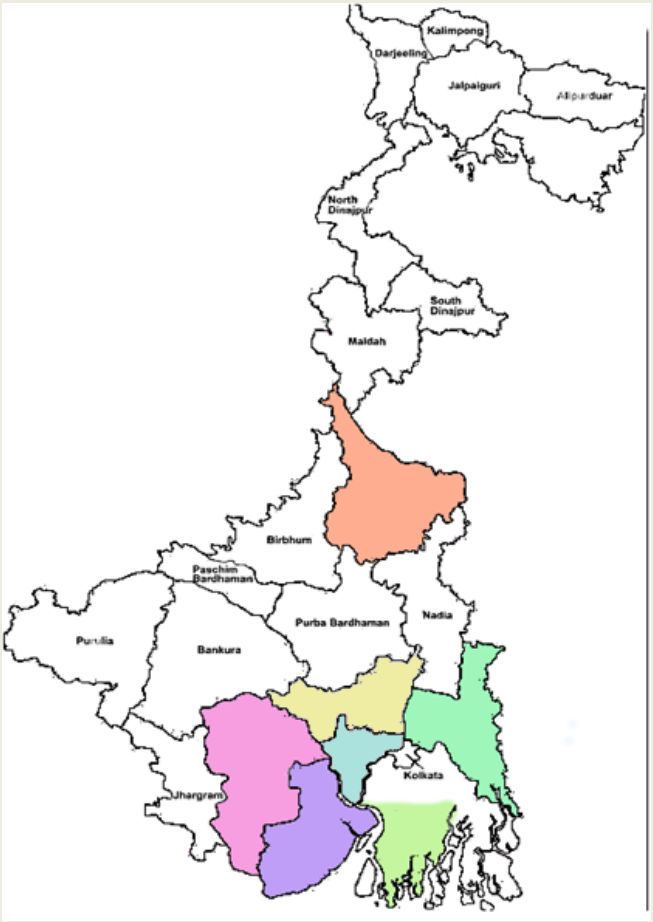


Figure 4.1: Map of West Bengal showing screening area.

Table 4.1: District wise screening in West Bengal

District	No. of Camps	Total No. of Samples
South 24 Parganas	38	6083
North 24 Parganas	03	446
East/West Midnapur	06	1572
Hooghly	04	383
Howrah	01	91
Murshidabad	01	101
Total	56	8676

A total of 8676 venous blood samples were collected from unrelated individuals from six districts (Table 4.1): mainly from South Bengal (6083). Participants were selected from different gram panchayats. Gram Panchayats in South Bengal such as Mathur, Noorpur and Khurdo of Diamond Harbour Block I, Block-II, East and West Midnapur (1572), North Parganas (446), Hooghly (383), Howrah (91) and Murshidabad (101). The number of camps organized, and the total number of participants was tabularized in Table 4.2.

Table 4.2: Number of Camps and participants for screening

Sl No	Date of Camp	Place of Camp	No. of Samples	NESTROFT Suspected	BTT	HbAE	HbEE	Others
1	06.08.2005-23.11.2005	Gobindapur Kalicharan High School (Ramnagar)	407	134	25	23	1	3
2	06.09.2005-09.12.2005	Bhawanipur Vivekbharati Vidyapith (Noopur)	242	83	23	19		1
3	25.11.2005 - 01.02.2006	Noopur High Madrasa (Noopur)	112	40	8	8	0	5
4	07.02.2006/03.03.2006/10.03.2006	Thakur Anukul Ch Junior High School (Moyrapada)	156	69	9	6	0	0
5	08.01.2006	Anubhab Welfare Society (Haora)	28	5	2	0	0	0
6	16.03.2006	Maheswara Priyanath Junior Hiigh School, (Noopur)	129	64	19	8	0	4
7	23.03.2006	Village Parjana, GP Mathura Block DH II, S. 24 Parganas	69	19	3	9	0	1
8	04.06.2006	Village Mankhand Block DH II, S. 24 Parganas	120	35	7	4	0	0
9	11.06.2006	Village Kadaibedia, South 24 Parganas	77	18	2	4	0	1
10	18.06.2006	Mathur J M High Schhool	105	32	9	4	0	0
11	26.06.2006	Village Gobindapur, South 24 Parganas	114	44	14	5	0	1
12	18.07.2006	Khardanala Bipin Behari Siksha Sadan	492	125	48	9	1	3
13	15.09.2006	Mathur Jatiram Memorial High School	441	129	30	13	2	0
14	01.02.2007	Satminsha High Schhol South 24 Parganas	121	47	8	8	0	2
15	19.02.2007	Marigachi High School H.S. South 24 Parganas.	253	117	16	16	2	0
16	18.05.2007	Raynagar Khepanath Sunil Baran Poura Vidyalaya, DH Block I	375	167	40	8	0	3
17	19.06.2007	Asurali Gram Panchayat, South 24 Parganas	110	31	4	2	0	1
18	02.08.2007	Vivek Siksha (Seorahati)	103	23	5	3	0	
19	24.08.2007	Makhana High School (Falta)	225	117	15	14	0	1
20	28.08.2007	Ashu rali Gram Unnayan Parwashad, Asurali	216	69	4	0	0	2
21	07.10.2007	Village Kultukari, South 24 Parganas	89	62	8	13	1	0
22	12.10.2007	Fatepur Sreenath Institution, South 24 Parganas.	105	63	8	6	0	3
23	18.11.2007	Sarwasha High School, DH Block II, South 24 Parganas.	103	40	6	2	0	1
24	22.11.2007	Bidhan Chandra College Rwashra	232	26	11	3	0	0
25	01.02.2007	Tatini Girls School, Fatepur, Block Falta, South 24 Parganas	145	42	12	1	1	4
26	27.12.2007	Village Sitarampur, Kakdip, South 24 Parganas	175	33	14	5	1	0
27	06.01.2008	Nabagram Sevak Sangha, Nabagram	63	6	2	2	0	2
28	24.02.2008.	Khamarpukur Netaji Sangha	153	Not done	17	7	0	0
29	14.06.2008.	Bajitpur High School	204	-	2	0	1	1
30	27.08 08.	Khandalia High School	353	-	42	22	1	2
31	12.12.2008.	Parashurampur Sarbodya Sihka Sadan	123	-	6	3	0	0
32	16.12. 2008.	Pana K. C. High School	98	-	0	8	0	1
33	28.12.2008	Vivekananda Vidyamandir	220	-	21	4	1	0
34	14.01.2009	Chunabhati Village Camp	91	-	2	0	0	2

35	05.11.2009	Chanda High School	96	-	4	3	0	0
36	18.11.2009	Bhadura Girls High School	100	-	8	6	0	0
37	15.12.2009	Sundarika Girls High School	109	-	4	3	0	1
38	25.10.2010	Sangata Bhaumik Smriti Sanrakhan Samiti	62	-	3	1	0	0
39	25.01.2010	PG Govt.Inst. for Physical Education	253	-	7	4	0	1
40	31.03.2010	Narayantala Bidyabhavan High School	75	-	4	0	0	0
41	07.04.2010	Rwashra Institute (Science Club)	26	-			0	0
42	12.04.2010	Barkhatia High Madrasa	60	-	3	3	0	
43	18.11.2010	Geokhali Nikunja Memorial Girls High School	103	-	1	6	0	1
44	29.11.2010	Haldia Govt. Spons.Vivekananda Vidhyabhaban	416	-	13	6	0	0
45	23.10.2011	Mirpur Village Camp	147	-	5	0	0	1
46	09.02.2011	Mahwasadal Girls College	383	-	9	4	0	
47	20.01.12	Shjvnagar Mukshyada Sundari Vidyamandir	82	-	3	3	0	
48	20.04.2012	Kwashorenagar Sachindra Sikha Sadan	102	-	8	0	0	1
59	28.06.2012	Ajay Ananda Vidyamandir	104	-	1	0	0	
50	24.05.2013	Lalgola Sangibani	101	-	4	2	0	1
51	04.06.2013	Saptarshi Vivekananda Society	74	-	6	0	0	
52	28.11.2013	Basanti Devi College collaboration with Inner Wheel	67	-	5	3	0	1
53	10.02.2015	Howrah Sangha Adarsha Balika Vidyalaya	82	-	1		0	1
54	11.02.15	Jagatpur Rukmini Vudyalaya	75	-	2	2	0	
55	10.03.2015	Gorcha Second Lane Camp colla.c Inner Wheel Club	50	-	3	0	0	2
56	01.04.2015	Barwasha Sasibhusan Janakalyan Boy's High School	60	-	5	1		1
	Total		8676	1635	535	275	11	55

Cell Morphology

The following red blood cell morphology was observed and classified:

Size of RBC

Normocytic normal size
Microcytic smaller than normal
Macrocytic larger than normal
Anwasocytosis cells of various sizes present

Haemoglobin content

Normocromic normal colour and haemoglobinization
Hypochromic pale colour and lack of adequate haemoglobin

Shape

Poikilocyte abnormally shaped red cell
Ovalocyte oval red cell
Elliptocyte same as ovalocyte
Sickle cell or
Dense elliptocyte seen with Hbs and haemoglobinopathies
Tear drop pear shaped red cell.
Schistocyte cellular fragment
Keratocyte red cell shaped like a military helmate (helmate cell)

Spiculated red cells

Echinocyte	crenated red cell (regularly spiculated, usually reversible)
Spherocyte	round red cells, not biconcave
Stomatocyte	red cell mouth shaped area of central pallor
Target cell	area of central pallor with dense centre like "bullseye''

In suspected cases, the peripheral smears showed: normocytic cells; microcytic cells; hypochromic cells; Variation in size and shape (anisocytosis and poikilocytosis); and increased percentage of reticulocytes. Presence of Microcytic cells in the peripheral smear shows abnormality in the sample indicating thalassemia or any haemoglobinopathy.

NESTROFT

NESTROFT results for number of suspected cases in 27 camps and 4807 individuals was given in Table 4.2. A total of 1635 suspected cases were picked up from NESTROFT, and 595 abnormalities was confirmed through CBC and HPLC. When both the results were compared, the following observation was recorded (Table 4.3)

Table 4.3: Percentage distribution of NESTROFT

NSTROFT Results	No. of Samples	Percent
NESTROFT Positive	1635	34.01
NESTROFT Negative	3172	65.98
Confirmed Cases	595	12.39
NESTROFT False positive	1041	21.66

In the category of β Thalassaemia trait (Table 4.4), the frequency of NESTROFT positive, and NESTROFT negative and suspected cases were 77.356 percent, 1.89 percent and 20.75 percent, respectively. However, it was observed that NESTROFT was not so suitable test as a preliminary screening in the detection of haemoglobinopathies as false positive cases was 21.66 percent. It was observed that among the individuals with HbAE, the frequency of NESTROFT positive, and NESTROFT negative and suspected cases were 26.86 percent, 40.30 percent and 32.84 percent, respectively.

Table 4.4: Validation of NESTROFT (N=4807)

Types of haemoglobinopathy	NESTROFT Positive (N^+)	NESTROFT Negative (N^-)	Suspected ($N^\pm$)
BTT	77.36 percent	1.89 percent	20.75percent
HbAE	26.86 percent	40.30 percent	32.84percent
HbEE	100 percent	0 (0.00)	0
HPFH	33.33percent	0 (0.00)	66.67 percent
Normal	11.35percent	68.79percent	19.86percent

Key: N^+= NESTROFT Positive, N^- =NESTROFT Negative, $N^\pm$= NESTROFT Suspected, BTT= B Thalassaemia Trait, HbAE=HbE Heterozygous (HbE carrier) and HbEE= HbE Homozygous (HbE Patient) HPFH= Hereditary persistence of fetal haemoglobin (Carrier state),

In the case of HBEE, NESTROFT positive were 100 percent. On the other hand, among the individuals with HPFH (Hereditary persistence of fetal haemoglobin) carrier state, 33.33 percents were NESTROFT positive, 66.67 percents NESTROFT suspected cases and none of the individuals were found as NESTROFT negative. In the case of HPFH, one individual was NESTROFT positive, and two individuals were NESTROFT suspected. In the case of normal individuals where NESTROFT negatives were maximum (68.79 percents), NESTROFT positive were11.35 percents and suspected were 19.86 percents. NESTROFT eliminated the maximum number of normal individuals.

Cellulose Acetate Electrophoresis (CAM)

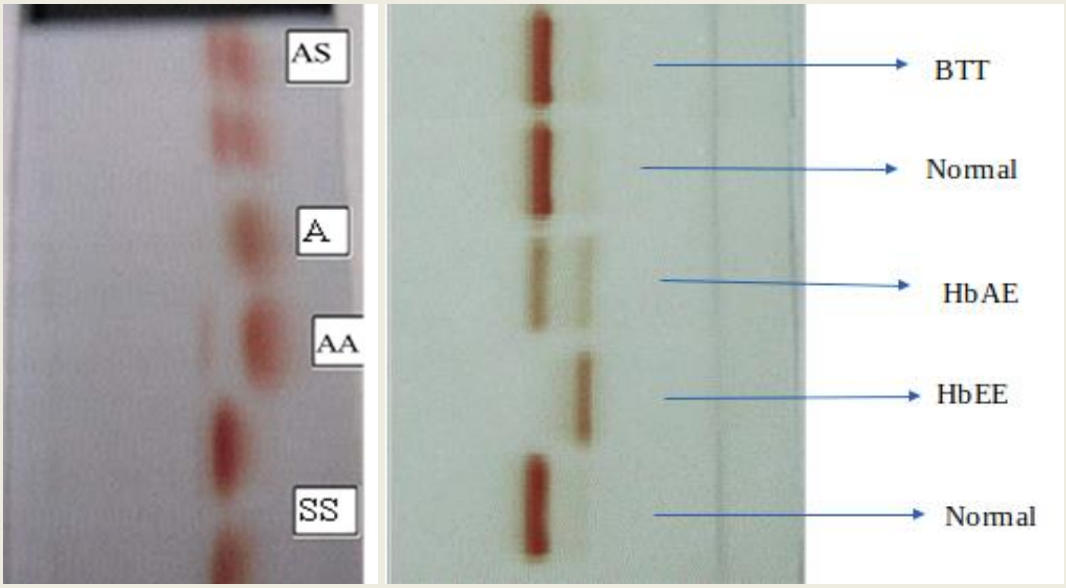


Figure 4.2: Haemoglobin Electrophoresis (I- Sickle Cell, II- Hemoglobin E & BTT)

Haemoglobin Electrophoresis involves the separation of several components of Haemoglobin (A_0, A_2 & F) according to their protein molecular weight and the respective electric charge potential. Keeping in view the different pattern of mobility of different components of haemoglobin in haemoglobin electrophoresis, various haemoglobinopathies could be identified (Figure 4.2).

Keeping in view the different pattern of mobility of different components of haemoglobin in haemoglobin electrophoresis, (Figure 4.2) suitable controls were run with the five samples. The general mobility (slow to fast) of different haemoglobin in alkaline pH (8.4 and 8.9) follows the pattern: HbA_2/HbE, HbS/HbD Punjab/HbQ India, HbF, HbA_0, HbH/HbBarts. With reference to these patterns, the samples were analyzed. In the suspected samples there was a consistent band at the position of HbA_0. In the band position of HbA_2/HbE was visible but less than that in HbA_0. When the bands at this position were compared in all the samples, it was observed that in other samples, the quantity of band at HbA_2/HbE was lesser than that of other sample HbAS and the highest concentration was in sample HbEE. In sample-HbAE there was another band confirming heterozygote HbE.

Complete Blood Count (CBC)

CBC values are a useful indicator for detecting abnormalities in the sample. The mean, SD and SE of all the parameters of CBC was given in Table 4.5. The data was arranged with the HPLC data when confirmed for BTT and HbAE.

Table 4.5. Statistical constants of haematological parameters and haemoglobin fraction among the individuals of HbE carrier, β Thalassemia carrier and normal person

0

Haematological parameter	BTT (n=535)			HbAE (n=275)			Normal n= (7800)		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
RBC (*10^{6}/µl)	5.9148	0.5997	0.0582	4.982	0.5110	0.0624	4.890	0.5046	0.0157
Hb(gm/dl)	10.7424	1.0530	0.1022	10.9139	1.3071	0.1597	11.95	1.3275	0.0414
Hct(percent)	36.4192	3.4880	0.3386	36.0023	3.8546	0.4712	38.6850	3.6508	0.1140
MCV (fl)	61.7919	3.6854	0.3578	72.2779	4.8886	0.5976	79.2657	5.7401	0.1793
MCH (pg)	18.2384	1.1853	0.1150	21.8797	1.8939	0.2315	24.5060	2.1728	0.0678
MCHC (gm/dl)	29.5066	1.4778	0.1434	30.2704	1.0944	0.1337	30.8960	1.0510	0.0328
HbA_2 (percent)	5.2090	0.5569	0.0540	31.4089	8.8106	1.0770	2.6970	0.3230	0.0100
HbF (percent)	0.7759	0.1262	0.0122	0.8128	0.6785	0.0829	0.1902	1.2494	0.0300

Validation of CBC cut-off against Mean and SD for Haematological parameters in derivation sub- sets.

The individuals who were finally predicted to have HbAE by the estimation of HbA_2 percent on the principle of HPLC and other individuals in the category of normal, HbEE and HPFH were also screened in the haemoglobin testing system. Mean RBC was higher in BTT (5.91) than HbAE (4.98) or Normal (4.89) indicating microcytosis. Therefore, in the study, the average value of Hb was 10.74 gm/dl, RBC was 5.91µl, MCV was 61.79 fl and MCH was 18.24 pg in case of β thalassemia. But in the case of HbAE, the values of RBC, Hb, MCV and MCH were 4.98µl, 10.91gm/dl, 72.27 fl and 21.88 pg, respectively.

Analyzing the values of CBC and the result of HPLC, it was observed that in the case of β thalassaemia trait (BTT), the complete blood count predicted 99.04 percent positive. Here CBC positive signifies MCV less than 70 fl, MCH less than 20 pg, haemoglobin was more than 11.5 gm/dl and number of RBC count was greater than 5.5*106/µl (Table 4.4). Prediction of HbEE from CBC was 100 percent correct. This stringent laboratory protocol ensures the reliable result. But in the case of the HbE trait where CBC negative was in higher percentage than CBC positive. This indicates that the samples have the value of MCV in the range of 70.5fl to 74.5fl, and the value of MCH within 20.6 pg to 23.5 pg. The mean of haematological parameters (CBC) and haemoglobin fraction are calculated among the individuals of β Thalassaemia carrier and normal person (Table 4.6)

Table 4.6: Percentage distribution of complete blood count (CBC).

Category	CBC Positive(C^{+})	CBC Negative(C^{-})	Total percentage
BTT	99.05 percent	0.94 percent	8.83 percent
HbAE	34.33 percent	65.67 percent	5.58 percent
HbEE	100 percent	0	0.17 percent
HPFH	0	100 percent	0.25 percent
Normal	6.45 percent	93.54 percent	85.16 percent

Key: CBC= complete Blood Count, C^{+}= Indicative of carrier status and C^{-}=non-indicative of carrier status. (Percentage within the bracket) C+= MCV<70±4 fl, MCH <20±4, RBC>$5.5*10^6$ and Hb<10gm/dl; C-=MCV>70fl, MCH>20pg and RBC<$5.5*10^6$,Hb>10gm/dl

Table 4.7: Statistical constants of haematological parameters (CBC) and haemoglobin fraction among the β Thalassemia carrier and normal person.

Haematological Parameter & Hb -fraction	Normal Individual (HbAA)			Suspected Cases			Different Statistical functions			
	X	SD	SE	X	SD	SE	t-value	df	p-value	Remarks
RBC	4.96	0.52	0.01	5.89	0.66	0.03	38.40	6972	0.0001	Significancant
Haemoglobin	12.40	1.51	0.18	11.09	1.48	0.06	19.07	6972	0.0001	Significancant
MCV	79.82	6.04	0.07	62.79	7.11	0.31	61.08	6973	0.0001	Significancant
MCH	25.10	2.48	0.31	19.05	3.73	0.16	51.21	6972	0.0001	Significancant
HbA_2	2.71	0.32	0.03	5.07	0.66	0.03	135.75	4637	0.0001	Significancant
HbF	0.18	1.79	0.03	0.75	1.03	0.04	7.12	4637	0.0007	Significancant

Table 4.7 gives the mean and SD values calculated for all the indices of RBC, the percentage value of HbA_2 and the percentage of HbF. In the conclusion, where the CBC value was evaluated, the HbA_2 and HbE are more important parametric roles in detecting carrier status. This was important for detection of the thalassaemia carrier status and other abnormal haemoglobin variant status. Suspected cases showed the high red blood cell count (5.89±0.66), low mean values of MCV and MCH, 62.79 and 19.05 respectively. Significant association was observed between Hb, MCV, MCH. HbA_2 and HbF. t- test results of significance between RBC of normal and BTT individuals shows t=61.08 and P-value was less than 0.0001indicating statistical significance, t- test results of significance between MCV of normal and BTT individuals shows, t=38.40 and P-value was less than 0.0001indicating statistical. t- test results of significance between MCH of normal and BTT individuals shows, t=51.21 and P-value was less than 0.0001indicating statistical significance in all the parameters

High-performance liquid chromatography (HPLC)

Phenotypes of Haemoglobinopathy variants detected by HPLC among the screened participants is given in table 4.8. Respective chromatogram gave peaks of Hb A_0, A_2, and HbF along with S-window.

Table 4.8: Frequency distribution of phenotypes of Haemoglobinopathies /abnormalities detected among the tested participants.

Phenotypes	N (%)
B- Thalassaemia trait	535 (6.16)
HbAE	275(3.16)
HbEE	11(0.13)
HbAS	3
HbES	1
E-β Thalassaemia	1
Delta-β Thalassaemia	1
HPFH heterozygote	5 (0.05)
Mildly rawased HbF (1.0 - 5.0)	40 (0.46)
Moderately rawased HbF (5.0 - 10.0)	4 (0.04)
Total	876 (10.10)

The percentage of HbA2 value more than 3.5 in retention time 3.3–3.9 minutes, was taken as a β-thalassaemia carrier. When the percentage of HbA2 value was in the range of 23.2 to 35.5 in retention time 3.3–3.9 minutes in the chromatogram, which showed an addition of HbA2, HbE window was confirmed for HbE carrier status. S-window was observed at retention time of 4.30–4.70 minutes and HbF at 1.00–1.30 minutes. The total abnormalities detected and confirmed by HPLC in the present study was given in Table 4.6. The various abnormalities identified are B Thalassaemia Trait (6.16 percent), HbE carrier (3.16 percent), HPFH (Hereditary persistence of fetal haemoglobin) and HbEE (HbE homozygote), HbAS, one case of HbE- Sickle, one case of HbE- β thalassaemia and individuals with mild (0.46 percent) and moderately raised (0.04 percent) fetal haemoglobin (Figure 4.3).

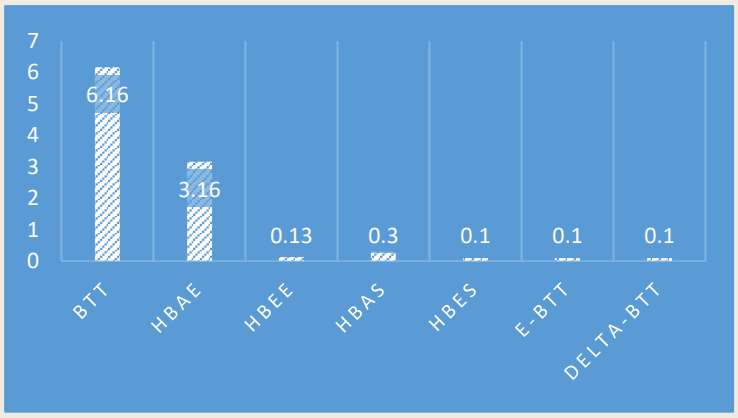


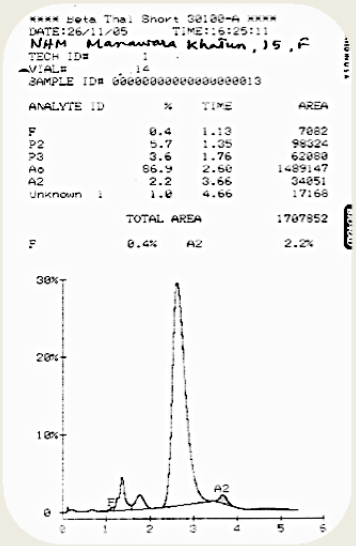
Figure 4.3: Bar Graph Showing Distribution of Haemoglobinopathy in Eastern Region.

876 (10.4 percent) abnormalities of the globin genes were recorded among the studied sample of which about three-fifth were due to β thalassaemia including β Thalassaemia trait and β Thalassaemia with HbAE. The second most common abnormality observed was HbAE which accounted for more than 3.16 percent of the total. HbAS was observed to be present at lower frequency. One case of HbAE interacting with HbAS was an important observation. The mother of the subject carried the HbS allele and was from Medinipur district of southeast West Bengal.

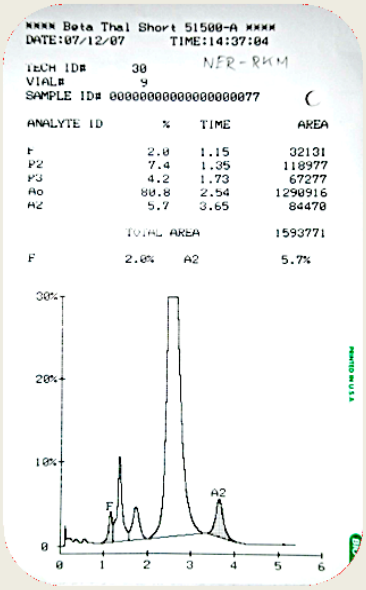
Five cases of HPFH were detected along with a very high number of subjects with mildly raised HbF (up to 0.46 %). Moderately elevated HbF level samples were considered for further analyses for any other molecular abnormality at the G>A β-cluster of the β-globin gene family.

Chromatograms of haemoglobinopathies.

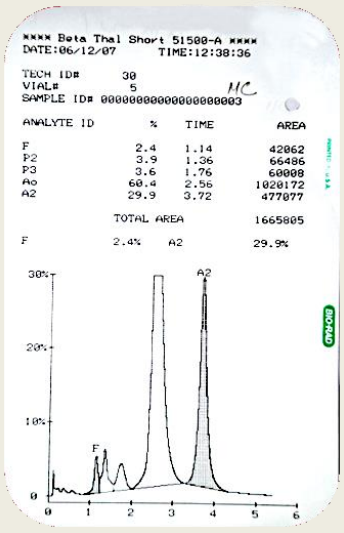
Figure 4.4 shows the chromatograms from HPLC. In the first chromatogram the percentage value of HbA_2 was 2.2 which had a retention time was 3.72 minutes was exhibited. The percentage value of HbF was 0.4 which had a retention time was 1.10 minutes. The peak value of P_2 and P_3 were 5.7 (RT=1.35 minute) and 3.6 (RT=1.76), respectively. The value of HbA or HbA_0 was 96.6 percent which had a retention time of 2.60 minutes. One unknown peak was shown which was negligible. According to the interpretation of the result of the chromatogram the value of the HbA_2 was less than 3.5 percent, predicting the normal individual. Therefore, the first chromatogram of the individual was normal.



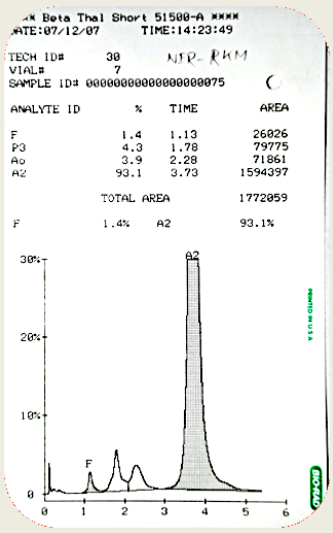
NORMAL



BTT



HbAE



HbEE

Figure 4.4: HPLC chromatograms of presumptive Normal, BTT, HbAE and HbE Homozygote

In the case of the second chromatogram HbA2 and HbF values were 5.7 percent and 2 percent respectively according to their retention time. The peak value of P2 and P3 were 7.4 percent and 4.2 percent respectively. This was shown in the β-thalassaemia trait sample as its HbA2 value was more than 3.5 percent. This sample was diabetic according to ADS (American Diabetic Society) and WHO.

The third chromatogram showed that special type of peak where HbA2 and HbE co-eluted with each other. In the case of HbE, HbA2 ranges generally from 18.5 percent to 36.0 percent. So, this sample was detected as an HbE carrier. Here fetal haemoglobin (HbF) value was 2.4 percent. The HbA value was 60.4 percent. The peak value of P2 and P3 were 3.9 percent and 3.6 percent, respectively. In the case of the fourth chromatogram which exhibited such a type of chromatogram where HbA2 and HbE co-eluted simultaneously that value was 93.1percent. This was the HbEE i.e. HbE homozygote state. Here HbF value was 1.4percent and P3 peak value was 4.3 percent. Based on HPLC, the haemoglobinopathy status among different communities in West Bengal was determined.

Community wise distribution of haemoglobinopathies

Table 4.9 presents the community-wise distribution of BTT, HbAE and HBEE phenotypes that was detected. 10.1 percent of subjects carried abnormalities related to haemoglobins. Of which 6.1% are β-thalassaemia traits and 4.27% are having the commonest structural haemoglobin variant, the HbE both in heterozygote and homozygote state (HbAE and HbEE).

Table 4.9: Distribution of thalassaemia and haemoglobinopathies by different communities

Name of the communities	Categories		Normal individual	percent	BTT	percent	HbAE	percent	HBEE	percent	others	suspected
	Male	Female										
Muslim N=1571	609	962	1401	89.18	76	4.83	74	4.71	3	0.19	17	1.08
Mahishya N=2482	1147	1335	2250	90.65	171	6.89	56	2.26	4	0.16	1	0.04
Poundra N=1171	612	559	1022	87.28	85	7.26	62	5.29	2	0.17	0	0
Bagdi N=180	88	92	153	85	23	12.78	3	1.67	1	0.56	0	0
Namasudra N=289	127	162	237	82.01	32	11.07	5	1.73	0	0	15	5.19
Chasi N=133	43	90	123	92.48	9	6.77	0	0	1	0.75	0	0
Brahman N=273	160	113	257	94.13	13	4.76	2	0.73	0	0	1	0.37
Rajbanshi N=117	74	43	101	86.32	0	0	16	13.68	0	0	0	0
Kaora N=139	74	65	128	92.09	11	7.91	0	0	0	0	0	0
Kayastha N=312	167	145	292	93.59	14	4.49	6	1.92	0	0	0	0
Chrwastian N=124	56	68	124	100	0	0	0	0	0	0	0	0
Others N=1885	856	1029	1712	90.82	101	5.36	51	2.71	0	0	21	1.11
Total 8676	4013	4663	7800	89.9	535	6.16	275	3.16	11	0.13	55	0.63

Table 4.9 presents the distribution of thalassaemia, HbAE, HbEE and other haemoglobinopathies among the different ethnic communities. Ten numerically dominant ethnic groups are identified residing for at least three generations in the area. The largest Hindu group among them was the Mahishya, which was also one of the most dispersed ethnic groups in the southern part of West Bengal. The other Hindu communities are Bagdi, Poundra Kshatriya, Kaibarta, Kaora and Namasudra.

Muslims are found to be mostly residing in the villages under Noorpur panchayat. Inter-community marriages are generally prohibited. It was noted that approximately 30 percent of the Muslims residing in these three panchayats have been converted from either Poundra or Namasudra groups in the recent generations. In the other category, the screened communities (Koibarta, Napit, Muchi, etc) having lower than 100 participants or those who could not reveal their ethnic identity, have been included.

In the present study, prevalence of β thalassaemia was high among the Bagdi community 12.78 percent. The Bagdi was an indigenous agrarian caste of West Bengal who, it was believed originated from Kshatriya father and Vaishya mother. (Risley 1891:37-43), followed by Namasudra 11.07 percent another community belonging to the lower strata in Bengal.

Molecular characterisation

A total of 535 DNA samples were sequenced for molecular characterization of the β globin gene. Table 4.10 specifies the spectrum of different Beta Thalassaemia mutations across the ethnic communities screened. Molecular characterization of β thalassemia mutation was done by ARMS PCR in earlier studies, to identify the common, known mutations. A total of 32 different mutations have been identified, in the present study. Though IVS 1-5 (G-C) mutation covered 68.1 percent β thalassemia mutation, other rare mutations have been identified. IVS1-5 (G-C), Cd30 G>C and Cd 41/42 (-TCTT) mutations, a four-base pair deletion in the human β-globin gene and covered 83.74 percent of the cases. There are extensive variations of β-thalassemia mutations in the population. Two deletions and one frame shift has been observed. Each of the identified mutation has been provided in Table 4.10 to enhance the diagnostic implications of finding a rare mutation to avoid the birth of thalassemia major.

Table 4.10: Distribution of different Beta Thalassaemia mutations across ethnic communities.

Sl. No.	Mutations	Ethnic Communities																			
		Bagdi	Brahman	Goala	Jugi	Kaibarta	Kaora	Kayastha	Khumbhakar	Mahishya	Muchi	Muslim	Namasudra	Napit	Poundra	Rajbanshi	Sadgope	Chasi	Teyar	Others	Total
1	619 bp Del	2								3											5
2	Ca+1 A>C									1											1
3	Cap+32 G>C			1											2						3
4	Cd 6 A>T														1						1
5	Cd 6 G>A									1											1
6	Cd 11 (– A)				1							2			1						4
7	Cd 15 +G	1																		2	3
8	Cd 15 G>T		1									2	4							1	8

9	Cd 16/17 +G											1								1
10	Cd 17 -A													1						1
11	Cd 2 C>T						1	2		1										4
12	Cd 23 G>T																		4	4
13	Cd 41/42 (-TCTT)	1	1	1			4	3	1	21	1	2		4	2		1		1	1	46
14	Cd 7 A>G									1			1								2
15	Cd 8/9 (+G)		1			1						1						1			4
16	Cd15 G>A						1					1		2					5	9
17	Cd2 C>T												3						1	4
18	Cd23 G>T,																	1		1
19	Cd30 G>C	1	1							9			5		1				21	38
20	Cd 88 C-T																		2	2
21	IVS I -I to IVS 1-27 Deletion	1																1	3	5
22	IVS II -I G>A														2					2
23	IVS II -16 G>C				1													1		2
24	IVS II -16 G>T CD54 Frame Shift									1						1		2	4	8
25	IVS II 75 G>T						1													1
26	IVS II 75 G>T									2								1		3
27	IVS1-34 C>G						1													1
28	IVS1-42 A>G														2					2
29	IVS1-5 G>C	17	9		1	2	8	9		129 (24.11)		67 (12.5	19	1	69 (12.9)	1	4		3	24	363 (68.1)
30	IVS1-5 G>T									2										2
31	IVS1-65 A>G,														2				1	3
32	IVS1-68 A>G														2				1	3
	Total	23	13	2	2	3	16	14	1	171	1	76	32	7	85	2	5	7	5	69	535

Five common β thalassaemia mutations that are predominantly observed especially in Eastern India are: IVS-I-5-G>C, Cd 8/9 +G, Cd 41/42 –TCTT, Cd30 G>C, and Cd 15 G>A) that are the dominant mutations detected in the study area.

IVS 1-5 (G-C) mutation was observed to be the most predominant mutation in the Eastern India and was accountable for 68.1 percent of the cases, across the ethnic communities studied. Codon 41/42 (–TCTT) mutation was found in 8.59 percent of the BTT screened. Cd 30 (G>C) was prominent among the Mahishya and was observed in 38 (7.1 percent) cases. It was reported that the origin of codon 30 (G > C) mutation was associated to United Arab Emirates (Rezaee, 2012). Codon 15 (G–A), an Asian Indian mutation that was also found in Syria and Israeli Arabs was observed among 9 cases. Codon 8/9 (+G) was the most frequent mutation which has similarity with those in the Northeast of Iran and was also an Asian Indian mutation, present in low frequency among the studied population. 619 bp deletion, an Indian mutation was found in five of the participants in the present study. The spectrum of β thalassaemia was wider and diverse among the Muslim, Poundra and the Mahishya etc.

The spectrum of beta thalassemia mutations in different ethnic groups is plotted and shown in Figure 4.5. A degree of similarity and significant variations was evident in the type and frequency of mutations when the present mutations profile was compared with various ethnic groups in the graphical representation.

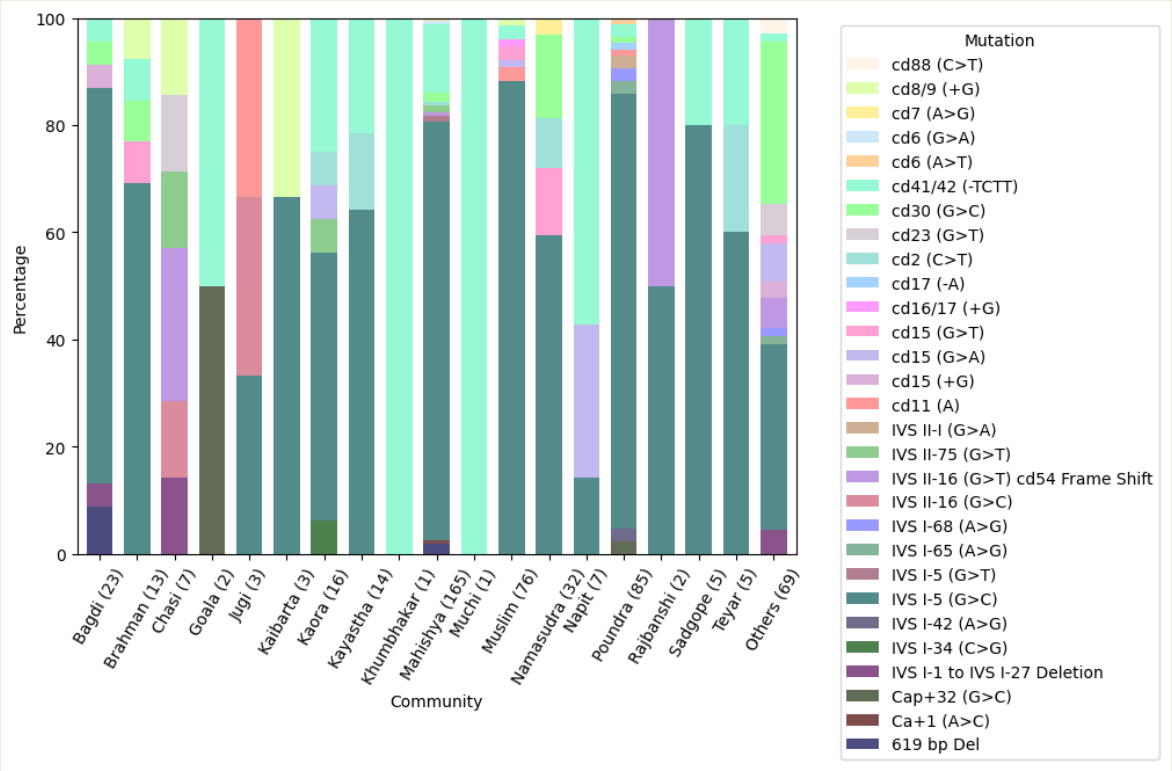


Figure 4.5. Spectrum of beta thalassemia mutations in different ethnic groups studied in India.

When compared with earlier study by Basu and Chakarvarty (2002) found similar frequencies from a large-scale follow-up study on the thalassaemia patients from West Bengal. Table 4.11 compares the result of the two studies.

Table 4.11. Frequency distribution of phenotypes of Haemoglobinopathies compared with earlier study.

Phenotypes	Present study	Basu and Chakravarty, 2002
	N (percent from total abnormality)	N (percent from total tested)
B-Thalassaemia trait	535 (6.16)	124(10.87)
HbAE	275(3.16)	37(3.24)
HbEE	11(0.13)	3(0.26)
HbAS	3 (0.03)	6(0.53)
HbES	1	0
E-β Thalassaemia	1	73(6.40)
β-Thalassaemia	1	1(0.09)
HPFH heterozygote	5 (0.05)	0
Mildly raised HbF (1.0 - 5.0)	40 (.46)	0
Moderately raised HbF (5.0 - 10.0)	4 (0.04)	0
B-Thalassaemia major	0	23(2.02)
Total abnormalities	876 (10.10)	269
Total Tested	8676	1141

Key: BTT= B Thalassaemia Trait, HbAE=HbE Heterozygous (HbE carrier) and HbEE= HbE Homozygous (HbE Patient) HPFH= Hereditary persistence of fetal haemoglobin (Carrier state).

The frequency of different phenotypes of β Thalassaemia are summarized and compared to that of the earlier published reports from West Bengal (Bandopadhyay et al 2004, Bandopadhyay et al 1999, Sengupta et al 2006) and data on all India studies (Vaz et al 2000) in Table 4.11. Out of these, the IVS-I-5-G>C mutation was accountable for about 68.1percent of cases in the present study (Figure 4.4). 619 bp deletion at 3' to β-globin gene was noted in about 3% of cases. 165 alleles with CD 26-G>A (HbE) were detected among a total of 535 chromosomes with HbE gene (not included with β thalassaemia mutations). CD 41/42 (-TCTT) mutation was found in 6 alleles (0.02) with a similar frequency as reported from West Bengal. The other less common mutations were CD 8/9 (+G) (0.004) and CD 15 (G>A) (0.004).

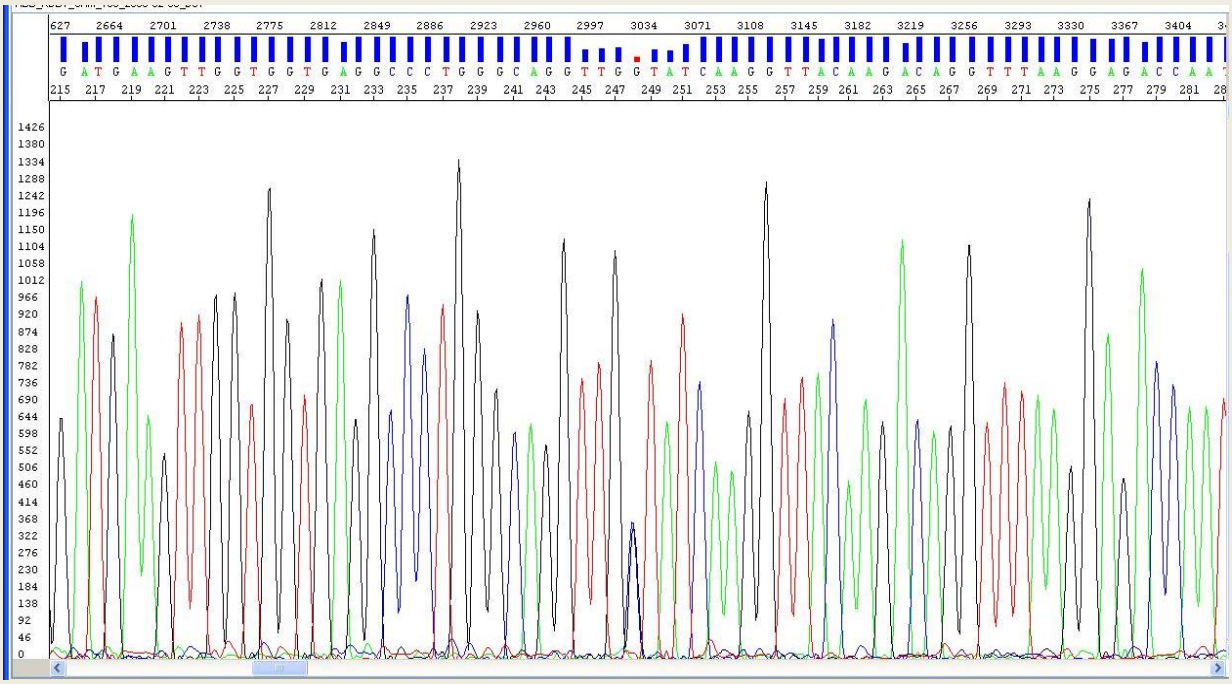


Figure 4.6: Mutation at IVS 1-5 (G>C)

Higher frequencies of these two mutations were reported earlier (Bandopadhyay et al 2004, Bandopadhyay et al 1999, Sengupta et al 2006), which may be due to inclusion of patients' homozygotes for these mutations in the samples analysed. Haemoglobin E was caused by substitution of Glutamic acid (Glu) for a Lysine (Lys) residue at codon no 26 of a β-globin gene (Figure 4.5). HbE trait has no clinical significance. A person with HbE trait was perfectly normal phenotypically but was severe with other haemoglobinopathy traits. HbS results from a single base-pair mutation in the β-globin gene on HbA. An adenine-to-thymine substitution (GAG→GTG) in the sixth codon replaces glutamic acid with valine in the sixth amino acid position of the β-globin chain located on the short arm (p-arm) of chromosome 11 (Figure 4.6).

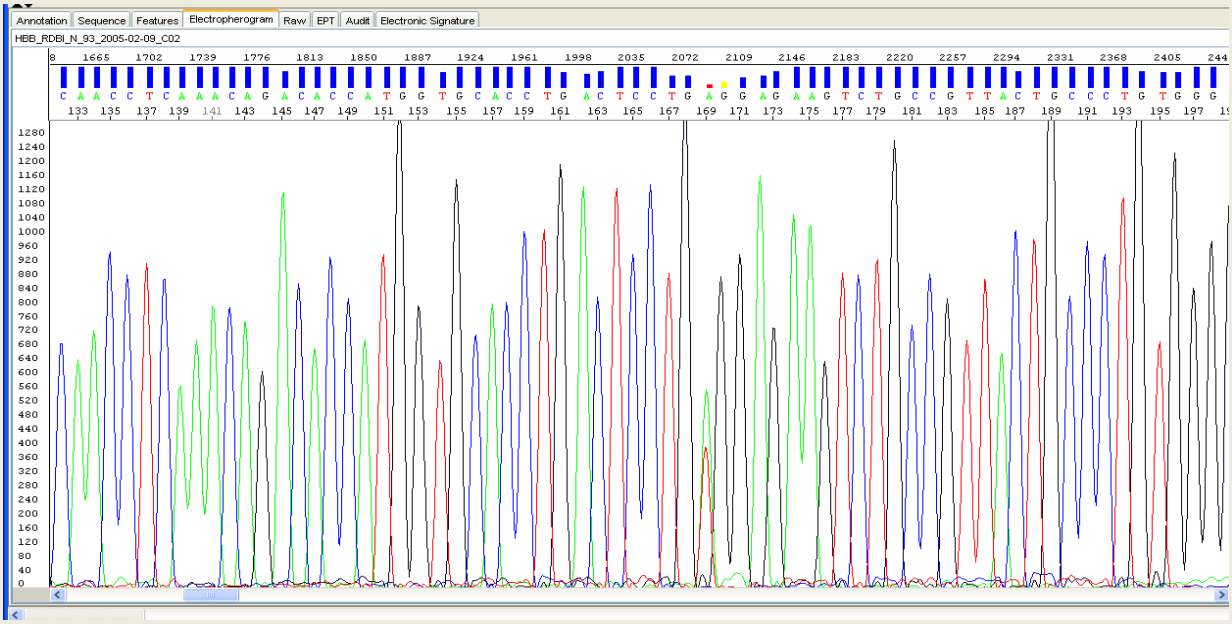


Figure 4.7: Mutation at Codon 26 (G>A) (HbAE)

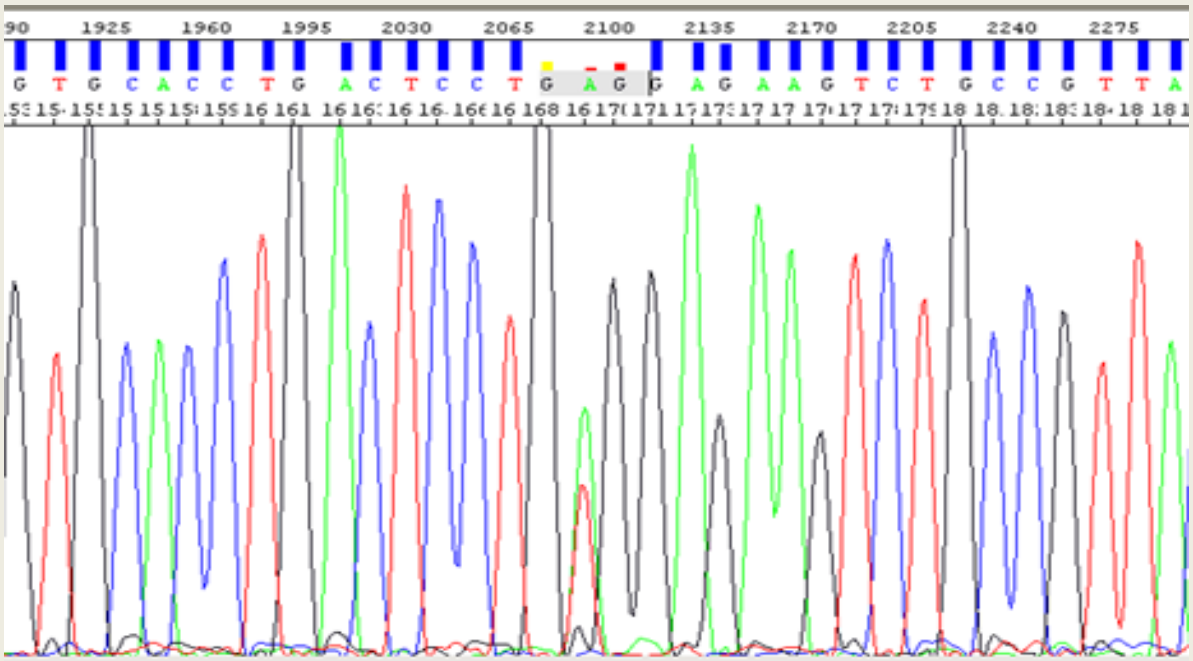


Figure 4.8: HbAS mutation at Codon 6

Chapter 05

HAEMOGLOBINOPHATHIES IN NORTHEAST REGION

The northeast region comprises of seven states, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura. Screening for haemoglobinopathy was conducted in Ri Bhoi District, Kamrup district, Kohima district, East Khasi Hills District, West Khasi Hills district and West Jaintia hills district. The total number of camps and the corresponding samples collected was given in Table 5.1 and dates in which the camps were held was given in Table 5.2.

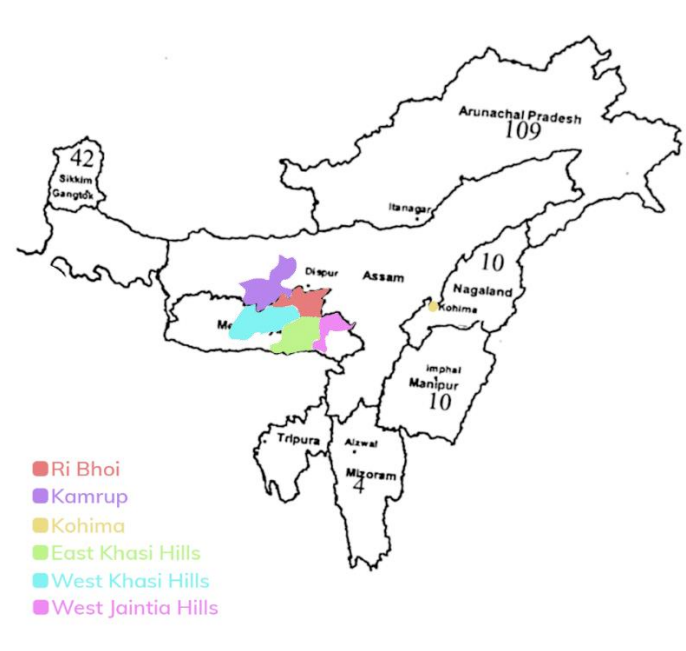


Figure 5.1: Map of Northeast India showing the districts where screening was conducted.

Table 5.1: Details of camps and samples in Northeast India.

District	No. of Camps	Total No. of Samples
Ri Bhoi District	4 Camps	410
Kamrup district	2 Camps	227
Kohima district	2 Camps	201
East Khasi Hills District	4 Camps	373
West Khasi Hills district	2 Camps	222
West Jaintia hills district	3 Camps	406

Table 5.2: Date wise Screening Camps in North East India.[3]

Sl.No.	Date of Camp	Place of Camp	No. of Samples	Abnormalities
1	17.10.2010	Amjok/ Umling	101	25
2	06.11.2010	Amkhang/ Umsning	100	20
3	10.03.2011	Alagjari/ BoKo	122	67
4	13.04.2011	Baghor Gaon/ Boko	105	58
5	14.06.2011	Zadima village	105	11
6	15.06.2011	Merima village	96	08

[3] No Solubility test was done at NERC.

7	09.05.2013	Pepba village	103	26
8	10.05.2013	Pepba village	100	16
9	14.07.2013	Tyrna village	147	03
10	09.08.2013	Tyniar village	23	02
11	08.03.2014	Darrang village	105	29
12	21.07.2014	Nartiang village	175	86
13	22.07.2014	Narting village	126	51
14	08.11.2014	Phillankata	105	53
15	09.11.2014	Phillankata	104	37
16	10.04.2015	Umdang	112	73
17	11.04.2015	Umdang	110	57
	Total		1839	524

Result and Discussion

A total of 1839 blood samples were collected from nine communities (War Khasi=170, Bhoi Khasi=201, Khynriam=203, Lyngngam=222, Garo=209, Pnar=301, War Jaintia=105, Rabha=227 and Angami Naga=201) for screening the haemoglobinopathies of Northeast India. The screening

Table 5.3: Frequency distribution of normal and suspected samples

Population	Normal (%)	Suspected (%)	Total
Bhoi Khasi	156 (77.61)	45 (22.39)	201
Khynriam Khasi	161 (79.31)	42 (20.69)	203
War Khasi	165 (97.06)	05 (02.94)	170
War Jaintia	76 (72.38)	29 (27.62)	105
Pnar	164 (54.49)	137 (45.51)	301
Garo	119 (56.94)	90 (43.06)	209
Lyngngam Khasi	92 (41.44)	130 (58.56)	222
Angami Naga	182 (90.55)	19 (09.45)	201
Rabha	102 (44.93)	125 (55.07)	227
Total	1315 (71.51)	524 (28.49)	1839

After screening (CBC), 524 (28.49%) individuals were identified as suspected cases, and the suspected cases were highest among the Lyngngum Khasi (58.56%) and lowest among the War Khasi (02.94%). The screening was based on RBC >5.00, MCV < 75 fl, MCH < 24 pg, HGB < 11 g/dl, MCHC=range 32g/dL to 36g/dL, HCT range in adult males 42% to 54% and adult women 38% to 46%.

The mean and SD value of RBC, HCT, MCV, Hb, MCH and MCHC parameters are shows in table-5.4 for normal and suspected individuals' blood samples. Blood samples analysis done by automated blood cell counter instrument community wise. The normal range of RBC was in male 4.7 to 6.1 million cells/ mcm (millions per cubic millimeter) or 4.0 to 5.9x10*12/L and in female,

it was 4.2 to 5.4million cells/mcm or 3.8 to 5.2x10*12/L. The normal range of HCT in males 0.40 -0.54 / 40-54% and in females 0.36 -0.46 / 36-46%. Haemoglobin normal level for males was 14 to 18 g/dl and in females was 12 to 16g/dl. The normal value of MCV ranges from 80fL to 100fL. The normal range for MCH was 27 to 31 picograms per cell. Normal levels of MCH are between 26 and 33 picograms (pg) of haemoglobin per RBC. A normal MCHC value was typically in the range between 32g/dL to 36 g/dL (320g/L to 360g/L).

Table 5.4: Mean value of RBC, HCt, MCV, Hb, MCH and MCHC of both Normal and Suspect CBC blood parameters population wise.

Population	Normal or suspect	No.	RBC mean±SD	HCt mean± SD	MCV mean± SD	Hb mean± SD	MCH mean±SD	MCHC mean ±SD
Bhoi Khasi	normal	156	4.55±0.98	42.04±9.42	92.77±8.82	13.57±3.17	29.90±3.57	32.24±1.64
	Suspect	45	4.89±0.86	32.38±5.63	66.67±6.72	9.89±2.34	20.09±2.73	30.18±2.76
Khynriam Khasi	normal	161	4.33±0.52	41.28±5.59	95.46±8.92	13.10±1.91	30.31±3.53	31.79±1.48
	Suspect	42	5.25±0.59	45.48±8.08	87.72±8.41	13.50±3.02	26.53±3.51	31.81±10.99
War Khasi	normal	165	4.51±2.27	40.87±7.97	92.35±12.39	13.21±5.42	29.45±3.53	31.42±2.06
	Suspect	05	4.44±0.91	38.46±10.16	86.28±10.63	11.94±3.59	26.59±4.47	30.70±1.96
Lyngngam Khasi	normal	92	4.62±0.47	41.43±4.68	89.89±7.01	12.94±1.45	28.00±2.37	31.21±1.40
	Suspect	130	5.41±0.53	41.37±8.02	76.21±10.66	12.79±2.48	23.47±3.33	30.89±1.56
War Jaintia	normal	76	4.06±0.59	36.25±5.55	89.53±5.60	11.41±1.76	28.19±2.64	31.53±2.07
	Suspect	29	4.99±0.67	40.99±5.99	83.04±7.99	12.37±2.52	24.74±2.92	29.92±2.90
Pnar/Syentang or Jaintia	normal	164	4.41±0.64	33.30±5.51	75.98±9.80	13.16±2.03	30.45±11.45	39.62±4.20
	Suspect	137	4.65±0.66	32.25±6.27	69.25±7.87	12.67±2.47	26.99±3.54	39.03±2.16
Garo	normal	119	5.10±0.55	42.18±5.16	82.94±6.29	13.10±1.63	25.70±2.26	31.03±1.35
	Suspect	90	5.41±0.77	35.42±5.73	64.78±7.50	11.28±1.89	20.90±2.67	31.78±1.42
Rabha	normal	102	4.12±0.35	38.31±2.99	93.53±8.21	12.31±1.02	30.00±3.01	32.10±1.21
	Suspect	125	4.82±0.85	35.23±5.44	78.04±11.57	11.19±1.75	24.72±3.82	31.74±1.33
Angami Naga	normal	182	4.21±0.39	41.25±3.33	98.28±6.14	13.02±1.20	30.95±2.29	31.50±0.89
	Suspect	19	5.21±1.62	39.82±7.63	85.38±11.50	12.33±2.91	26.19±4.36	30.63±1.84

The average mean value of Normal RBC for all nine populations was (4.434) with SD (0.316) and in the suspected cases the average mean value was (5.008) and SD was (0.340) respectively. The average mean value of normal HCT was 39.657 % and SD was 3.075 and in suspected cases average mean (37.933%) along with SD (4.454). Likewise, overall the mean value of MCV (90.81fL) with SD (6.806), normal Hb mean (12.89) with SD (0.639), suspected Hb mean value (11.99) with SD (1.072), mean value of MCH normal are (29.217) with SD (1.649) and suspected mean value (24.469), with S D (2.528) and normal MCMH mean (32.493), with SD (2.701) and suspected mean (31.853) along with S D (2.780) respectively.

Table 5.5: Frequency distribution of Haemoglobin among the nine communities

Population	Range of Haemoglobin								Total
	Very Low (<9.0)		Low (9.1 – 11.0)		Normal (11.1- 13.0)		High 13 >		
	No.	%	No.	%	No.	%	No.	%	
Bhoi	8	(3.92)	20	(9.80)	93	(45.59)	83	(40.69)	201
Khynraum	7	(3.46)	24	(11.88)	60	(29.70)	111	(54.95)	202
War Khasi	12	(7.16)	17	(10.00)	55	(32.35)	86	(50.59)	170

Lyngngam Khasi	19 (8.55)	86 (38.74)	90 (40.54)	16 (7.20)	211
War Jaintia	9 (8.57)	32 (30.48)	38 (36.19)	26 (24.76)	105
Pnar	8 (2.67)	31 (10.33)	110 (36.67)	151 (50.33)	300
Garo	24 (11.48)	103 (49.28)	76 (36.36)	6 (2.87)	209
Rabha	10 (4.41)	62 (27.31)	113 (49.78)	42 (18.50)	227
Angami Naga	4 (1.99)	15 (7.46)	86 (42.79)	96 (47.76)	201

Table 5.5 shows the frequency distribution of haemoglobin level among the nine communities of northeast India. It was found that haemoglobin level ≤11.0 category 60.76% of Garo in low Hb level followed by Lyngngum Khasi (47.29%), War Jaintia (39.05%) and Rabha (31.72%) than theirs normal and high category of Hb level. Whereas Angami Naga (9.45% 0, Pnar (13.00), Bhoi Khasi (13.72%), Khyriam Khasi (15.34%) and War Khasi (17.16%) individuals are in the very low and low category of haemoglobin range respectively. Haemoglobin level among the study population normal to high highest among the Angami Naga (90.55%), then Pnar (87.00%) followed by Bhoi Khasi (86.28%), Khyriam Khasi (84.65%), War Khasi (82.94%), Rabha (68.28%), War Jaintia (60.95%), Lyngngum Khasi (46.74%) and Garo (39.23%) respectively.

Table 5.6: Allelic frequency of Haemoglobin E in nine study populations of Northeast India.

Population /State	No	Haemoglobin Type/Genotype			E gene frequency
		AA	AE	EE	
War Khasi (Meghalaya)	170	165 (97.06)	5 (2.94)	00	0.0178
Bhoi Khasi (Meghalaya)	201	156 (77.61)	42 (20.90)	3 (1.49)	0.1194
Khynriam Khasi (Meghalaya)	203	161 (79.31)	40 (19.70)	2 (0.99)	0.1084
Lyngngam Khasi (Meghalaya)	222	92 (41.44)	78 (35.14)	52 (23.42)	0.4099
Garo (Meghalaya)	209	118 (56.46)	59 (28.23)	32 (15.31)	0.2943
Pnar/ Jaintia (Meghalaya)	301	263 (87.38)	38(12.62)	00	0.0631
War Jaintia (Meghalaya)	105	76 (72.38)	28 (26.66)	1 (0.95)	0.1429
Rabha (Assam)	227	102 (44.03)	114 (50.22)	11 (4.85)	0.2996
Angami Naga (Nagaland)	201	182 (90.55)	17 (8.46)	2 (0.99)	0.0522

Note: (Figures in parentheses indicate percentage values).

The high frequency of HbE found among the Lyngngam (0.4099), Rabha (0.2996) and Garo (0.2943) followed by War Jaintia (0.1429), Bhoi Khasi (0.1194), Khyriam Khasi (0.1084), Pnar/ Jaintia (0.0631), Angami Naga 0.0522) and War Khasi (0.0178) respectively. Systematic population studies concerning the distribution of abnormal haemoglobin E in Northeast India (Das et. al., 1971; Flatz et al., 1972; cf. Deka et. al., 1988) shows that the hypothesis of a correlation between high prevalence of HB E in Austro-Asiatic speaking populations.

It was suggested that HbE was spread to Northeast India by Austro-Asiatic migration in the prehistoric past (Deka et. al., 1988). However, high frequency of Hb E among Tibeto- Burman

populations of Assam in subsequent studies (Das et. al., 1975, 1980; cf. Deka et. al., 1988) led to reconsideration of this proposal. Considerably high frequency of Hb E in the Rabha (0.2996) found in the present study corroborate this finding. In this respect the view of Deka et. al., (1988) can be mentioned herewith: "Reasons for the high frequency of the Hb E gene are possibly the cumulative effect of selection and normal physical fitness of its carriers". Thus, high incidence of Hb E in these population groups in the present study may be due to founder effect or local inbreeding and migration.

Table 5.7: Distribution of E-β Thalassemia mutation in Northeast India.

SL NO.	Sample ID	Phenotype	Mutation	
1	Bhoi 017	HbE	cd2 T>C	Normal βthal
2	Bhoi 032	HbE	cd2 T>C	Normal βthal
3	Bhoi 042	HbE	Cd 2 T>C& cd 26 GAG>AAG	
4	Rabha 001	HbEE	22 GAG>AAG	
5	Rabha 009	HbEE	22 GAG>AAG,cd2 CC	
6	Rabha 012	HbEE	22 GAG>AAG,cd2 CC	
7	Rabha 015	HbEE	22 GAG>AAG,cd2 CC	
8	Rabha 017	HbEE	22 GAG>AAG,cd2 CC	
9	Rabha 023	HbEE	22 GAG>AAG,cd2 CC	
10	Rabha 034	HbEE	22 GAG>AAG,cd2 CC	
11	Rabha 038	HbEE	22 GAG>AAG,cd2 CC	
12	Rabha 045	HbEE	22 GAG>AAG,cd2 CC	
13	Rabha 046	HbEE	22 GAG>AAG,cd2 CC	
14	Rabha 056	HbEE	22 GAG>AAG,cd2 CC	
15	Ang 010	HbEE	22 GAG>AAG,cd2 CC	
16	Ang 049	HbE	cd2 T>C	Normal β thal & HbE

The above Table shows the distribution of E β Thalassemia mutation of some present populations.

Table 5.8: Distribution of HbE of Northeast India

Population	No	AE (%)	EE (%)	Gene frequency	Source
Ahom	129	46.5	11.6	0.349	Flatz et. Al 1972
Ahom	125	41.6	10.4	0.304	Balgir,1993
Ahom	399	45.4	10.0	0.327	Das et al.1975
Ahom	119	47.1	16.8	0.403	Das et al.1988
Karbi (Mikir)	131	27.5	6.1	0.198	Deka et al.1980
Karbi	110	29.1	8.2	0.227	Das et.al.,1988
Boro Kachari	131	47.3	31.3	0.549	Deka et al.,1980
Boro Kachari	110	38.2	45.4	0.645	Deka et al.1988
MechKachari	124	44.4	31.5	0.536	Balgir,1993
Sonowal kachari	158	50.0	31.6	0.562	Balgir,1993
Sonowal	555	49.7	26.1	0.510	Das et al.,1975
Sonowal	106	45.3	17	0.396	Deka et al.1988
Mwashing	25			0.600	Do
Chutiya	62	46.8	6.4	0.298	Do
Rajbanshi	164	39.6	15.2	0.350	Das et al.,1980

Rajbanshi	102	36.3	4.9	0.230	Deka et al,1988
Garo	135	37.0	31.1	0.496	Das et al,1980
Rabha	128	55.5	25.8	0.535	Do
Lallung(Tiwa)	114	50.9	19.3	0.447	Do
Tiwa	27	25.9	18.5	0.315	Balgir,1995
Sut	22	4.6	0.0	0.023	Do
Gallong	108	35.19	3.7	0.2	Urade, 2014
Caucasoid Group					
Brahmin	98	10.2	2	0.051	Deka et.al,1988l
Kalita	104	19.2	1.9	0.115	Do
Kaibarta	101	24.8	1.0	0.134	Do
Kaibarta	124	20.2	18.5	0.286	Balgir,1991 b
Muslim	104	19.2	1.0	0.101	Das etal,1980
Assamese Sikh	15	20.0	20.0	0.300	Balgir,1995
Assamese(U.Ass)	182	15.9	2.8	0.107	Flatz et al,1972
Assamese(L.Assa)	133	19.5	3.8	0.135	Das et al,1980
Tea garden lab	336	0	13.1	0.067	Balgir,1993
RengmaNaga	148	1.4	0.0	0.007	Saha,1990
Urban Naga	65	3.1	0.0	0.015	Saha&Tay,1990
Rural Naga	83	0.0	0.0	0.000	Do
Hmar	81	2.4	0.0	0.012	Do
Naga	44	6.8	0.0	0.035	Balgir,1991a
Khasi	80	38.7	2.5	0.219	Das et al,1971
Khasi	120	36.4	4.3	0.225	Flatz et al,1972
Khasi	157	4.5	0.0	0.022	Saha,1990
Bodo	24	16.7	29.1	0.375	Saha,1990
War Khasi	170	5 (2.94)	0.0	0.0178	Present study
Bhoi Khasi	201	42 (20.90)	3 (1.49)	0.1194	Present study
Khynriam Khasi	203	40 (19.70)	2 (0.99)	0.1084	Present study
LyngngamKhasi	222	78 (35.14)	52 (23.42)	0.4099	Present study
Garo	209	59 (28.23)	32 (15.31)	0.2943	Present study
Pnar/ Jaintia	301	38(12.62)	0.0	0.0631	Present study
War Jaintia	105	28 (26.66)	1 (0.95)	0.1429	Present study
Rabha	227	114 (50.22)	11 (4.85)	0.2996	Present study
Angami Naga	201	17 (8.46)	2 (0.99)	0.0522	Present study

It was observed that the results of the present study in Northeast India indicate the highest frequency of Hb E among the Mishing (0.6000), which was followed by the Boro-Kachari (0.5934), Rabha (0.5352), Kachari (0.5069) and the Garo (0.4963). The Lyngngam (0.4099), Rabha (0.2996) and Garo (0.2943) of the present study are characterized with high frequency of Hb E. Among the others a low frequency of Hb E was found.

Discussion

Northeast India is represented by three major linguistic families, Austro-Asiatic, Indo-European and Tibeto-Burman. This wide-ranging linguistic diversity influences the genetic profile of the ethnic communities of the Northeastern states. The Austro-Asiatic group is reported to have a linguistic relation with the Mon-Khmer people of the mainland Burma as well as Thailand and the Khasis of Meghalaya is an example of the same (Sikdar, 2016). Findings of the present study indicate that the nine ethnic populations, War Khasi (Meghalaya), Bhoi Khasi (Meghalaya), Khynriam Khasi (Meghalaya), Lyngngam Khasi (Meghalaya), Garo (Meghalaya), Pnar/ Jaintia (Meghalaya), War Jaintia (Meghalaya), Rabha (Assam), and Angami Naga (Nagaland), studied vary among each other in terms of the frequency of HbE gene. Though prevalence of this gene was in low among these groups, two Austro-Asiatic populations namely the Lyngngam and Garo and one Tibeto Burman group namely the Rabha shows considerably high prevalence of HbE gene.

The high incidence of HbE gene in these population groups of the present study may be due to founder effect or local inbreeding, or probably due to the cumulative effect of natural selection and normal physical fitness of its carriers also plays important role behind this populations. HbE occurs due to a single point mutation in the β- chain. At position 26 of the there is a change in the amino acid, from glutamic acid to lysine. Although the HbE gene alone does not cause significant complications and do not require any treatment, its interactions with other haemoglobinopathies produce severe syndromes. Studies show HbE act as protective force in malaria endemicity and is established in various population groups of Northeast India. Therefore, the existence of dynamic interaction within these alleles in such a malarial environment is in existence. The only way to prevent a homozygous child to be born is to avoid having children with someone who is also a carrier of haemoglobin E.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Anthropological Survey of India

Chapter 06

KAP (Knowledge, Attitude, Practice) ABOUT THALASSAEMIA IN RURAL WEST BENGAL

Study area, sample and sampling

This study was conducted in Diamond Harbour II, South-24 Parganas of West Bengal. The sample included various settlements of people. Altogether, 2493 (males and females) sample respondents of the above 13-year-old age group from two clusters of villages, i.e., exposed and relatively unexposed, were contacted for the knowing their awareness level about Thalassemia. The selection of subjects was done very carefully so that subjects from varied backgrounds are covered.

The subjects were selected purposively. They were called individually and interacted. However, the queries were not merely confined to the schedule; rather, as a matter of fact, prior to going through the schedule, a comprehensive conversation was undertaken to thoroughly understand their family background and subsequently evaluate their eligibility to become an ideal subject.

For descriptive information about the socio-cultural background and features of the respondents, an interview was arranged, whereas a schedule was used to quantitatively measure the knowledge, practice, and attitude of the general population about Thalassemia. It was asked whether the respondent heard the name Thalassaemia, and if they gave a response 'yes' then only the total schedule was administered to evaluate their attitude and practice.

The study variables

1. Demographic data: The demographic questions included information on gender, educational qualification, age, caste, family structure, etc.
2. Awareness status about Thalassaemia. To assess the awareness status of Thalassaemia a structured schedule has been used.

Table 6.1: Description of Psychological Tools

	Description of Psychological Tools		
Name of Tools	Variables	Number of Items	Range of Scores
A- General Introductory Schedule	Name, Age, Gender, Education, Occupation, Marital status, Religion, Monthly Income, Type of Family, Total No. of Family Members, No. of brothers and Sisters, Sibling Position, Mother Tongue, About Father, About Mother, health related information etc.	16	
B- Thalassaemia Awareness Schedule	a) Information about prevalent diseases in the locality b) Knowledge about Thalassaemia c) Attitude towards Thalassaemia d) Social Distance e) Application f) Recommendation regarding awareness about Thalassaemia	06 15 06 06 10 05	 13-39 04-24 04-24 10-20 03-09

As the attitudes were measured based on a scaling technique similar to Likert scale, scoring was accordingly made. Maximum score was given for positive answer and minimum for negative answer in descending order.

Analysis of the data

The data were tabulated, classified and analyzed according to the aims of the present research and were put in the tabular form. Analysis of data has done into three parts- Part-I for exposed village, Part-II for relatively unexposed villages and Part-III considered as comparative analysis of both the clusters.

Part-I: Analysis of the Data (Exposed Villages)

In this cluster, five villages, namely Gobindpur Pashchim, Goanara, Mathur, Maheshwara and Ashapur have been selected. All these five villages come under the Diamond Harbour-II Block of South-24 Parganas district of West Bengal.

Demographic Analyses of the Sample

Table 6.2: Demographic Characteristics of the Sample (N=1251)

Sl.No	Characteristics	Category	Number	Percentage
1-	Gender	Male	661	52.837
		Female	590	44.764
2-	Age	13 to 25 years	337	26.938
		26 to 45 years	661	52.837
		45+ & above	253	20.223
3-	Education	Illiterate	192	15.347
		Can sign	103	8.233
		Primary(I-V)	264	21.103
		Secondary (VI-X)	532	42.525
		Higher Secondary (XI-XII)	72	5.755
		Graduate & others	88	7.034
4-	Occupation	Farmer	37	2.957
		Business	114	9.112
		Service	144	11.510
		Housewife	438	35.011
		Student	98	7.833
		Labour (including agricultural & daily wage)	386	30.855
		Unemployed	34	2.717
5-	Marital Status	Married	995	79.536
		Unmarried	234	18.705
		Others	22	1.758
6-	Type of Family	Joint	444	35.491
		Nuclear	807	64.508

Awareness about Thalassaemia

Table 6.3: Information about Prevalent Diseases in Locality

Sl.No.	Statements	Answer			
1-	General diseases in village	Cold, Cough, Diarrhoea, Fever, Malaria, Gastric, Asthma, Arthritis			
2-	General diseases among children	Cold, Cough, diarrhoea, Fever, Jaundice, Pox, Acidity			
3-	Have seen children to whom blood was given frequently	Yes 459(36.69) 414(33.093) *		No 253(20.223) 109(8.713) *	Can't say 6(.479) 10(.799)*
4-	Number of patients seen	0-Number 263(21.023) 106(8.47)*	1-Number 265(21.183) 216(17.266) *	2-Number 141(11.270) 101(8.073)*	3 & more 49(3.916) 100(7.993)*
5-	Was it transmitted by parents	Yes 48(3.836) 105(8.393)*		No 520(41.566) 357(28.537)*	Can't say 150(11.99) 71(5.675)*
6-	Knowledge about genetic diseases	Yes 240(19.184) 273(21.822)*		No 395(31.574) 223(17.825)*	Can't say 83(6.634) 37(2.957)*

Majority of respondents reported that cold, cough, diarrhoea, fever, gastric, asthma and arthritis are the general diseases people suffer from, in the village. The general diseases that children suffer from in the village, are cold, cough, diarrhoea, fever, jaundice, pox and acidity. Most people (69.783 percent) said that they have seen/ known about a child who was given blood frequently. Although they were familiar with genetic diseases, like- asthma, majority of them did not have any idea that it was a genetic disease which transmits from parents to offspring.
Out of 1251, only data were collected from 533 respondents on the level of awareness about Thalassaemia as only they had informed that they heard about Thalassemia before.

Table 6.4: Knowledge about Thalassaemia

Statements	Answer	
	Yes	No
Heard Thalassaemia word	533 (42.605)	718 (57.394)
Symptoms	Blood Deficiency, Blood transfusion, Fever, cold	
Sources (from where knew about it)	An.S.I organized health camp. &School Television & Health Centre Other sources	

Statements	Yes	No	Can't say
Awareness about cause	210 (39.399)	178 (33.395)	145 (27.204)
It was an inherited disease	226 (42.401)	138 (25.891)	169 (31.707)
It was a blood disorder	296 (55.534)	112 (21.013)	125 (23.452)
Knowledge about types of Thalassaemia	50 (9.380)	344 (64.540)	139 (26.078)
Names of those types	19 (3.564()	387 (72.607)	127 (23.827)
Knowledge about Thalassaemia carrier	104 (19.512)	230 (43.151)	199 (37.335)
A normal person can be a Thalassaemia carrier	152 (28.517)	124 (23.264)	257 (48.217)

Treatment required for Thalassaemia carrier	137 (25.703)	114 (21.388)	282 (52.908)
Any physical illness/ abnormalities shown by Thalassaemia carrier	163 (30.581)	63 (11.819)	307 (57.598)
Any mental illness/ abnormalities Thalassaemia carrier	153 (28.705)	62 (11.632)	318 (59.662)
Any mental disability/ abnormalities among the parents of Thalassaemia patients	154 (28.893)	85 (15.947)	294 (55.159)
Thalassaemia was treatable but not curable	194 (36.397)	111 (20.825)	228 (42.776)

From Table-6.3 it was concluded that less than 50 percent of respondents had heard 'Thalassaemia' as a disease (42.605 percent; 295 males & 238 females). However, only 15 percent of respondents answered about the symptoms of thalassaemia. Most of them said that fever, blood deficiency, blood transfusion and cold are the basic symptoms of Thalassaemia. While 42.401percent of them knew that it was an inherited disease, 55.53 percent have knowledge that it was a disorder of blood. Fifty respondents (9.38 percent) knew about two types of Thalassaemia. Most of them were within the age range of 26 years to 45 years. However, about 20percent of respondents knew about Thalassaemia carrier and about 29percent have knowledge that Thalassaemia carrier was a normal person, but only 19 respondents could tell the name of the two types of Thalassaemia.

When enquired about the source of knowledge, majority of respondents responded that they learnt from blood collection camp organized by Anthropological Survey of India (57.29 percent), 28.47 percent from television and health centre and 14.23 percent from others. It shows that the blood collection camps of AnSI was reasonably effective in disseminating the knowledge about the diseases.

Table 6.5: Knowledge about Thalassaemia by socio-demographic differences

Statements	Category	Variables	Answer
			Yes
1Heard Thalassaemia word	Gender	Male	295(23.581)
		Female	238 (19.024)
	Generation	13-25 years	182(14.548)
		Up to 45 years	264(21.103)
		45+ & above	87(6.954)
	Literacy	Illiterate	69(5.515)
		Literate	464(37.090)

Table 6.6: Attitude towards Thalassaemia

Statements	Strongly Agree	Agree	Disagree	Strongly Disagree
A thalassaemia child was born due to sin committed by parents	62 (11.632)	126 (23.639)	90 (16.885)	255 (42.213)
I feel uneasy to sit with a person, who was suffering from thalassaemia	129 (24.202)	126 (23.639)	139 (26.078)	139 (26.078)
The life becomes miserable for entire family, where anyone was suffering from thalassaemia	124 (23.264)	191 (35.834)	165 (30.956)	53 (9.943)
The family where someone was suffering from thalassaemia was unlucky	144 (27.016)	211 (39.587)	136 (25.515)	42 (7.879)

It was better to die than live with a deadly disease like; thalassaemia	145 (27.204)	159 (29.831)	136 (25.515)	93 (17.448)
I try to avoid a thalassaemia patient	64 (12.007)	181 (33.958)	196 (36.772)	92 (17.260)

Majority of respondents strongly disagree (42.21 percent) that a thalassaemic child was born due to sin committed by parents, and only 11.63 percent of respondents strongly agreed to such a view. While 24.20 percentage of respondents strongly expressed that they feel uneasy to sit with Thalassaemia patients, another 23.639 too held the same feeling though less intensively. Rest of the respondents either strongly disagree or simply disagreed to such feelings.

That life becomes miserable for the entire family if any of the family members suffers from Thalassaemia was the strong opinion of 23.26 percent of respondents. Another more than two thirds, agreed to the same less intensively.

27.01 percent of respondents strongly agreed that a family with someone suffering from Thalassaemia was unlucky, another 39.58 percent too agreed to the same. A strong negative attitude that it was better to die than live with a deadly diseases like Thalassaemia, was expressed by 27.20 percent; another 29.83 too agreed to such a view though not very strongly. However, about 54 percent of respondents strongly disagreed that they try to avoid a thalassemia patient.

Table 6.7: Attitude towards Thalassaemia by demographic attributes

Statements	Characteristics	Category	Strongly Agree	Agree	Disagree	Strongly Disagree
A Thalassaemia child was born due to sin committed by parents	Gender	Male	37 (12.542)	66 (22.372)	53 (17.966)	139 (47.188)
		Female	25 (10.504)	60 (25.210)	37 (15.549)	116 (48.739)
	Generation	13-25years	15 (8.241)	37 (20.329)	42 (23.076)	88 (48.351)
		Up to 45 years	38 (14.393)	70 (26.514)	36 (13.636)	120 ((45.454)
		45+ & above	9 (10.344)	19 (21.839)	12 (13.793)	47 (54.022)
	Literacy	Illiterate	11 (15.942)	20 (28.985)	20 (28.985)	18 (26.086)
		Literate	51 (10.991)	106 (22.844)	70 (15.086)	237 (51.077)
I feel uneasy to sit with a person, who was suffering from Thalassaemia	Gender	Male	68 (3.050)	74 (25.084)	69 (23.386)	84 (28.474)
		Female	61 (25.630)	52 (21.848)	70 (29.411)	55 (23.109)
	Generation	13-25years	51 (28.021)	50 (27.472)	47 (25.824)	34 (18.681)
		Up to 45years	59 (22.348)	60 (22.727)	83 (31.439)	62 (23.484)
		45+ & above	19 (21.839)	16 (18.390)	9 (10.344)	43 (49.425)

	Literacy	Illiterate	20 (28.985)	13 (18.840)	19 (27.536)	17 (24.637)
		Literate	109 (23.491)	113 (24.353)	120 (25.862)	122 (26.293)
The life becomes miserable for entire family, where anyone was suffering from Thalassaemia	Gender	Male	71 (24.067)	101 (34.237)	86 (29.152)	37 (12.542)
		Female	53 (22.268)	90 (37.815)	79 (33.193)	16 (6.694)
	Generation	13-25years	42 (23.076)	62 (34.065)	60 (32.962)	18 (9.890)
		Up to 45 years	57 (21.590)	103 (41.287)	76 (28.787)	28 (10.606)
		45+ & above	25 (28.735)	26 (29.885)	29 (33.333)	7 (8.045)
	Literacy	Illiterate	13 (18.840)	29 (42.028)	20 (28.985)	7 (10.144)
		Literate	111 (23.922)	162 (34.913)	145 (31.25)	46 (9.913)
The family where someone was suffering from Thalassaemia was unlucky	Gender	Male	81 (27.457)	113 (38.305)	81 (27.457)	20 (6.779)
		Female	63 (26.470)	98 (41.176)	55 (23.109)	22 (9.243)
	Generation	13-25years	44 (24.175)	71 (39.010)	53 (29.120)	14 (7.692)
		Up to 45 years	74 (28.030)	106 (40.151)	62 (23.484)	22 (8.333)
		45+ & above	26 (29.885)	34 (39.080)	21 (24.137)	6 (6.896)
	Literacy	Illiterate	19 (27.536)	34 (49.275)	15 (21.739)	1 (1.449)
		Literate	125 (26.939)	177 (38.146)	121 (26.077)	41 (8.836)
It was better to die than live with a deadly diseases like; Thalassaemia	Gender	Male	79 (26.779)	94 (31.864)	73 (24.745)	49 1(6.610)
		Female	66 (27.731)	65 (27.310)	63 (26.470)	44 (18.487)
	Generation	13-25years	46 (25.274)	57 (31.318)	53 (29.120)	26 (14.285)
		Up to 45 years	70 (26.515)	80 (30.303)	57 (21.590)	57 (21.590)
		45+ & above	29 (33.333)	22 (25.287)	26 (29.885)	10 (11.494)
	Literacy	Illiterate	23 (33.333)	13 (18.840)	17 (24.637)	16 (23.188)
		Literate	122 (26.293)	146 (31.465)	119 (25.646)	77 (16.594)
I try to avoid a Thalassaemia patient	Gender	Male	34 (11.525)	109 (36.949)	91 (30.847)	61 (20.677)
		Female	30 (12.605)	72 (30.252)	105 (44.117)	31 (13.025)

	Generation	13-25years	24 (13.186)	72 (39.560)	54 (29.670)	32 (17.582)
		Up to 45 years	28 (10.606)	92 (34.848)	105 (39.772)	39 (14.772)
		45+ & above	12 (13.793)	17 (19.540)	37 (42.528)	21 (24.137)
	Literacy	Illiterate	17 (24.637)	8 (11.594)	29 (42.028)	15 (21.739)
		Literate	47 (10.129)	173 (37.284)	167 (35.991)	77 (16.594)

Table 6.8: Attitude towards Thalassaemia by socio-demographic attributes

Statements	Characterwastics	Category	Very much difficult	Difficult	Little bit difficult	Not at all difficult
Living with a person who was suffering from Thalassaemia	Gender	Male	18 (6.101)	52 (17.627)	76 (25.762)	149 (5.508)
		Female	15 (6.302)	58 (24.369)	60 (25.210)	105 (44.117)
	Generation	13-25years	6 (3.296)	37 (20.329)	40 (21.978)	99 (54.395)
		Up to 45 years	21 (7.954)	55 (20.833)	74 (28.030)	114 (43.181)
		45+ & above	6 (6.896)	18 (20.689)	22 (25.287)	41 (47.126)
	Literacy	Illiterate	3 (4.347)	27 (39.130)	16 (23.188)	23 (33.333)
		Literate	30 (6.465)	83 (17.887)	120 (25.862)	231 (49.784)
Making friendship with a person who was suffering from Thalassaemia	Gender	Male	41 (13.898)	74 (25.084)	65 (22.033)	115 (38.983)
		Female	46 (19.327)	71 (29.831)	43 (18.067)	78 (32.773)
	Generation	13-25years	26 (14.285)	42 (23.076)	32 (17.582)	82 (45.054)
		Up to 45years	51 (19.318)	72 (27.272)	58 (21.969)	83 (31.439)
		45+ & above	10 (11.494)	31 (35.632)	18 (20.689)	28 (32.183)
	Literacy	Illiterate	18 (26.086)	20 (28.985)	20 (28.985)	11 (15.942)
		Literate	69 (14.870)	125 (26.939)	88 (18.965)	182 (39.224)
Feeling discomfort with a neighbour, who was suffering from Thalassaemia	Gender	Male	36 (12.203)	67 (22.711)	71 (24.067)	121 (41.016)
		Female	28 (11.764)	53 (22.268)	61 (25.630)	96 (40.336)
	Generation	13-25years	21 (11.538)	33 (18.131)	48 (26.373)	80 (43.956)
		Up to 45 years	32 (12.121)	65 (24.621)	70 (26.515)	97 (36.742)

		45+ & above	11 (12.643)	22 (25.287)	14 (16.091)	40 (45.977)
	Literacy	Illiterate	6 (8.695)	21 (30.434)	24 (34.782)	18 (26.086)
		Literate	58 (12.500)	99 (21.336)	108 (23.275)	199 (42.887)
Working with a person whose any family member was suffering from Thalassaemia	Gender	Male	51 (17.288)	30 (10.169)	50 (16.949)	164 (55.593)
		Female	33 (13.865)	21 (8.823)	43 (18.067)	141 (59.243)
	Generation	13-25years	32 (17.58)	14 (7.692)	36 (19.780)	100 (54.945)
		Up to 45 years	42 (15.909)	29 (10.984)	36 (13.636)	157 (59.469)
		45+ & above	10 (11.494)	8 (9.195)	21 (24.137)	48 (55.172)
	Literacy	Illiterate	10 (14.492)	6 (8.695)	15 (21.739)	38 (55.072)
		Literate	74 (15.948)	45 (9.698)	78 (16.810)	267 (57.543)
Establishing marriage relation in a family where someone was suffering from Thalassaemia	Gender	Male	190 (64.406)	60 (20.338)	26 (8.813)	19 (6.440)
		Female	151 (63.445)	57 (23.949)	22 (9.243)	8 (3.361)
	Generation	13-25years	115 (63.186)	42 (23.076)	15 (8.241)	10 (5.494)
		Up to 45 years	172 (65.151)	57 (21.590)	23 (8.712)	12 (4.545)
		45+ & above	54 (62.068)	18 (20.689)	10 (11.494)	5 (5.747)
	Literacy	Illiterate	34 (49.275)	26 (37.681)	7 (10.144)	2 (2.898)
		Literate	307 (66.163)	91 (19.612)	41 (8.836)	25 (5.387)
Accepting a life partner having Thalassaemia trait/carrier	Gender	Male	184 (62.372)	74 (25.084)	23 (7.796)	14 (4.745)
		Female	158 (66.386)	55 (23.109)	15 (6.302)	10 (4.201)
	Generation	13-25years	121 (66.483)	47 (25.824)	9 (4.945)	5 (2.747)
		Up to 45 years	170 (64.393)	59 (22.348)	22 (8.333)	13 (4.924)
		45+ & above	51 (58.620)	23 (26.436)	7 (8.045)	6 (6.896)
	Literacy	Illiterate	45 (65.217)	16 (23.188)	6 (8.695)	2 (2.898)
		Literate	297 (64.008)	113 (24.353)	32 (6.896)	22 (4.741)

Table 6.9: Practices relating to the thalassemia

Statements	Yes	No
Do you try to adopt recommended improved practices in many spheres of life?	417(78.236)	116(21.763)
Have you ever talked about the benefit of adoption of such improved practices?	335(62.851)	198(37.148)
Have you ever helped any one to follow such improved practices?	269(50.469)	264(49.530)
Do you practice what you have learned about diseases?	303(56848)	230(43.151)
Are you the first to adopt any improved practices regarding diseases in your village?	5(0.938)	528(99.061)
Did you ever discuss with your family members about Thalassaemia?	212(39.774)	321(60.225)
Have you tested your blood for Thalassaemia?	107(20.075)	426(79.924)
Have you tested blood of your family members for Thalassaemia?	81(15.196)	452(84.803)
Have you attended any awareness programme related to Thalassaemia?	143(26.829)	390(73.170)
Do you think that family level counselling was effective to combat these diseases?	465(87.242)	68(12.757)

Regarding adaptation of recommended improved practices and informing others about the benefit of such improved practices 78.26 percent and 62.85 percent of respondents, respectively expressed their willingness about it, but the opposite picture was noticed regarding blood testing for Thalassaemia for themselves and for their family members. Only 20.07 percent and 15.19 percent of respondents tested blood for themselves and for their family members, respectively for identification of the Thalassaemia trait. Though only 39.77 percent of respondents expressed that they discussed the matter regarding Thalassaemia with their family members 87.24 percent of respondents feel that family-level counselling was effective to combat these diseases.

Table 6.10: Gender and Awareness about Thalassaemia

Aspects of TAS	Grouped compared	Mean	SD	t- value
Knowledge about Thalassaemia	Male	26.354	2.828	2.920**
	Female	25.805	1.414	
Attitude towards Thalassaemia	Male	15.151	2.828	.892NS
	Female	15.175	2.756	
Social Distance Thalassaemia	Male	14.621	3.021	1.356NS
	Female	14.952	2.621	
Practice	Male	14.395	2.128	.315NS
	Female	14.347	1.414	

** Significant at .01 Level; NS Not Significant

Aspects of TAS	Mean	SD
Knowledge about Thalassaemia	26.564	5.606
Attitude towards Thalassaemia	15.161	3.032
Social Distance Thalassaemia	14.769	3.007
Practice	14.373	2.197

The sample depicted that irrespective of age, education and occupation there was no significant difference between males and females, so for as knowledge, attitude and social distance about Thalassaemia because the t-values are not significant even at .05 level i.e., .723, .286, .700 and .272 respectively.

Table 6.11: Generation gap and Awareness about Thalassaemia

Aspects of TAS	Grouped compared	Mean	SD	f-value
Knowledge about Thalassaemia	13-25 years	24.761	2.828	4.879*
	26-45 years	26.080	4.949	
	46 & above	25.620	1.823	
Attitude towards Thalassaemia	13-25 years	15.316	3.435	2.039NS
	26-45 years	15.166	1.414	
	46 & above	14.491	1.414	
Social Distance Thalassaemia	13-25 years	15.027	5.078	1.159NS
	26-45 years	14.825	1.706	
	46 & above	14.569	1.707	
Practice	13-25 years	14.508	1.054	2.749NS
	26-45 years	14.264	1.717	
	46 & above	14.425	2.121	

* Significant at .05 Level; NS Not Significant.

Table 6.10 revealed that irrespective of sex and education significant difference was noticed among the three groups, so far as knowledge, attitude, social distance and practice are concerned F-values are 4.879, 2.039, 1.159 and 2.749 respectively only the knowledge aspect was significant at 0.05 level. But if we see the mean values, it was clear with the increase of age not only knowledge increases but also attitudes change in a positive direction, which ultimately helps to minimize the social distance for different social issues.

Table 6.12: Education and Awareness about Thalassaemia

Aspects of TAS	Merged for all villages	Mean	SD	t-value
Knowledge about Thalassaemia	Illiterate	25.147	7.071	1.401NS
	Literate	26.394	5.639	
Attitude towards Thalassaemia	Illiterate	15.478	3.535	.779NS
	Literate	15.114	4.242	
Social Distance Thalassaemia	Illiterate	15.565	2.121	3.206NS
	Literate	14.648	2.828	
Practice	Illiterate	14.014	1.011	3.057NS
	Literate	14.436	1.414	

NS Not Significant

The above table shows that education has a quite good impact on knowledge about Thalassaemia, but it may not be a significant causative factor for positive attitude towards Thalassaemia and social distance about it. In this regard, we can say that proper family counselling was very much

essential for the improvement of attitude towards Thalassaemia and to minimize the social distance between the thalassaemic traits and normal person.

Table 6.13: Recommendation regarding Awareness about Thalassaemia

Statements	Answer		
	Very much effective	Much effective	Less Effective
How much effective would be the community level awareness programme to combat this problem?	329 (61.726)	164 (30.769)	40 (7.504)
If yes, how it can be organized? a-at local level (Panchayat) b-at area level (Block) c-at bigger network level/city level (District) d-at state level e-at national level	285 (53.470) 76(14.821) 43(8.067) 80(15.009) 49(9.193)		
Do you think that any awareness programme at the national level was more effective?	Yes 272 (51.031)	No 165 (30.956)	Can't Say 96 (18.011)
Do you think that any person can contribute something to the solution of this problem?	Yes 273 (51.219)	No 118 (22.138)	Can't Say 142 (26.641)
What will be your role? i. Convey message to others about it ii. Blood should be tested before marriage iii. Participation in such type of awareness programme iv. No comments			

Regarding recommendations, majority of respondents (89.36 percent) expressed that a community-level awareness programme would be efficient means to address social issues relating to thalassemia.

Relatively unexposed villages

In this cluster four villages i. e, - Khagrakona, Gagankolia, Channa and Sagra was selected for the data collection. all the four villages came under the Diamond Harbour-II Block of South-24 Parganas district of West Bengal.

Demographic characteristics of the sample respondents

Table 6.14: Demographic characteristics of the total Sample respondents

Sl. No.	Characteristics	Category	Number	Percentage
1-	Gender	Male	641	51.61
		Female	601	48.38
2-	Age	13 to 25 years	374	30.11
		26 to 45 years	636	51.20
		45+ & above	232	18.67
3-	Education	Illiterate	191	15.37
		Can sign	98	7.89
		Primary(I-V)	320	25.76
		Secondary (VI-X)	549	44.20

		Higher Secondary (XI-XII)	46	3.70
		Graduate & others	38	3.05
4-	Occupation	Farmer	76	6.11
		Business	87	7.00
		Service	29	2.33
		Housewife	335	26.97
		Student	155	12.47
		Labour (including agricultural & daily wage)	535	43.07
		Unemployed	25	2.01
5-	Marital Status	Married	941	75.76
		Unmarried	271	21.81
		Others	30	2.41
6-	Type of Family	Joint	571	45.97
		Nuclear	671	54.02

Table 6.15: Information about prevalent diseases in locality

Sl.N.	Statements	Answer			
1-	General diseases in village	Cold, Cough, Diarrhoea, Fever, Malaria, Gastric, Asthma, Arthritis			
2-	General diseases among children	Cold, Cough, Diarrhoea, Fever, Jaundice, Pox, Acidity			
3-	Have seen children to whom blood was given frequently	Yes- 540(43.478) 228 (18.357) *		No- 144 (11.594) 3 (24.154) *	Can't say- 329(26.489) NIL*
4-	Number of patients seen	0-Number- 268 (21.578) 11 (.885)*	1 Number 497(40.016) 120 (9.661)*	2- Number 145(11.674) 61(4.911)*	3 & more 101 (8.132) 39(3.14)*
5-	Was it transmitted by parents	Yes- 81(6.521) 111 (8.937)*		No - 764(61.513) 105(8.454)*	Can't say - 166(13.365) 15(1.207)*
6-	Knowledge about genetic diseases	Yes - 351(28.26) 141(11.352) *		No - 654(52.657) 87(7.004) *	Can't say - 6(.483) 3(.241) *

It was observed by the above Table 6.14 that most respondents opine that cold, cough, diarrhoea, fever, gastric, asthma and arthritis are the general diseases people suffer from, in the village. Regarding what are the general diseases children suffer from in the village, most of the respondents responded, i.e., cold, cough, diarrhoea, fever, jaundice, pox and acidity. Most people (61.835 percent) said that they have seen/ known about a child whose blood was given frequently. Although 39.612 percent of respondents were familiar with genetic diseases, like- asthma, TB, cancer etc. Only 15.458 percent knew that the child whose blood was given frequently was a genetic disease and it was transmitted by parents.

Table 6.16: Knowledge about Thalassaemia

Statements	Answer		
	Yes	NO	
Heard Thalassaemia word	231 (18.59 percent)	1011 (81.40percent)	
Symptoms	Weakness, yellow eye, big stomach, blood deficiency etc.		
Sources (from where knew about it)	TV, Poster, NGOs and Camp organised By Anthropological Survey of India		
Statements	Yes	NO	Can't say
Awareness about it	109 (47.18)	54 (23.376	68 (29.437)
It was an inherited disease	123 (53.24)	57 (24.675)	51 (22.077)
It was a blood disorder	192 (83.11)	27 (11.688)	22 (9.523)
Knowledge about types of Thalassaemia	13 (5.62)	126 (54.545)	92 (39.826)
Names of those types	4 (1.73)	130 (56.277)	97 (41.991)
Knowledge about Thalassaemia carrier	31 (13.41)	113 (48.917)	87 (37.662)
A normal person can be a Thalassaemia carrier	21 (9.09)	48 (20.779)	162 (70.129)
Treatment required for Thalassaemia carrier	15 (6.49)	47 (20.346)	169 (73.160)
Any physical illness/ abnormalities shown by Thalassaemia carrier	15 (6.49)	40 (17.316)	176 (76.19)
Any mental illness/ abnormalities Thalassaemia carrier	7 (3.03)	37 (16.017)	187 (80.652)
Any mental disability/ abnormalities among the parents of Thalassaemia patients	13 (5.62)	105 (45.454)	113 (48.917)
Thalassaemia was treatable but not curable	132 (57.14)	32 (13.852)	67 (29.004)

It was revealed that only 18.59 percent of respondents had heard the word Thalassaemia, but only 5 percent of respondents answered about the symptoms of Thalassaemia. Most of them said that fever, blood deficiency, blood transfusion and cold are the basic symptoms of Thalassaemia. Among those who heard the word 'Thalassaemia', 53.24 percent knew that it was an inherited diseases and 83.11 percent had knowledge that it was a disorder of blood. The rest of the respondents did not know about the genetic diseases.

Only 13 respondents (5.62 percent) between the age range of 13-18 years old knew that there are mainly two types of Thalassaemia. However, they knew about carriers that Thalassaemia was a normal person, but only four respondents (1.74 percent) could name them. When it was asked source of their knowledge, majority of the respondents informed about NGOs, television and health centres and very few responded from blood collection camp organized by the Anthropological Survey of India.

Table 6.17: Knowledge about Thalassaemia (item-wise bi-furcation) N= 231

Statements	Category	Variables	Answer
			Yes
Heard Thalassaemia word	Gender	Male	131(56.709)
		Female	100(43.290)
	Generation	13-25years	105(45.454)
		Up to 45 years	96(41.558)
		45+ & above	30(12.987)
	Literacy	Illiterate	19(8.225)
		Literate	212(91.774)

Statements	Category	Variables	Yes	No	Can't say
Awareness about it	Gender	Male	61(46.564)	32(24.427)	38(29.007)
		Female	48(48)	22(22)	30(30)
	Generation	13-25years	45(42.857)	33(31.428)	27(25.714)
		Up to 45 years	52(54.166)	18(18.75)	26(27.083)
		45+ & above	12(4)	3(10)	15(5)
	Literacy	Illiterate	6(31.578)	2(10.526)	11(57.894)
		Literate	3(1.415)	52(24.528)	57(26.886)
It was an inherited disease	Gender	Male	71(54.198)	30(22.900)	30(22.900)
		Female	52(52)	27(27)	21(21)
	Generation	13-25years	65(61.904)	25(23.809)	15(14.285)
		Up to 45 years	42(43.75)	26(27.083)	28(29.166)
		45+ & above	16(53.333)	6(20)	8(26.666)
	Literacy	Illiterate	7(36.842)	5(26.315)	7(36.842)
		Literate	116(54.716)	52(24.528)	44(20.754)
It was a blood disorder	Gender	Male	100(76.335)	17(12.977)	14(10.687)
		Female	82(82)	10(10)	8(8)
	Generation	13-25years	84(80)	13(12.380)	8(7.619)
		Up to 45 years	76(79.166)	9(9.375)	11(11.458)
		45+ & above	23(76.666)	4(13.333)	3(10)
	Literacy	Illiterate	16(84.210)	1(5.263)	2(10.526)
		Literate	166(78.301)	26(12.264)	20(9.433)
Knowledge about types of Thalassaemia	Gender	Male	10(7.633)	64(48.854)	57(43.511)
		Female	3(3)	62(62)	35(35)
	Generation	13-25years	6(5.714)	68(64.761)	31(29.523)
		Up to 45 years	4(4.166)	2(2.083)	90(93.75)
		45+ & above	1(3.333)	10(33.333)	19(63.333)
	Literacy	Illiterate	1(0)	5(26.315)	14(73.684)
		Literate	12(5.660)	122(57.547)	78(36.792)
Names of those types	Gender	Male	2(1.526)	70(53.435)	59(45.038)
		Female	2(2)	60(60)	38(38)
	Generation	13-25years	1(.952)	72(68.571)	32(30.476)
		Up to 45 years	2(2.083)	47(48.958)	47(48.958)
		45+ & above	1(3.333)	11(36.666)	18(60)
	Literacy	Illiterate	0(0)	2(21.052)	15(78.947)
		Literate	4(1.886)	126(59.433)	82(38.679)
Knowledge about Thalassaemia carrier	Gender	Male	21(16.030)	60(45.801)	50(38.167)
		Female	10(10)	53(53)	37(37)
	Generation	13-25years	23(21.904)	56(53.333)	26(24.761)
		Up to 45 years	6(6.25)	46(47.916)	44(45.833)

		45+ & above	2(6.666)	11(36.666)	17(46.666)
	Literacy	Illiterate	0(0)	4(21.052)	15(78.947)
		Literate	31(14.622)	109(51.415)	72(33.962)
A normal person can be a Thalassaemia carrier	Gender	Male	14(10.687)	27(20.610)	90(68.702)
		Female	7(7)	21(21)	72(72)
	Generation	13-25years	16(15.238)	21(20)	68(64.761)
		Up to 45 years	1(1.014)	21(21.875)	74(77.083)
		45+ & above	4(13.333)	6(20)	20(66.666)
	Literacy	Illiterate	0(0)	3(15.789)	16(84.210)
		Literate	21(9.905)	45(21.226)	146(68.867)
Treatment required for Thalassaemia carrier	Gender	Male	8(6.106)	27(20.010)	96(73.282)
		Female	7(7)	20(20)	73(73)
	Generation	13-25years	10(9.523)	27(25.714)	68(64.761)
		Up to 45 years	2(2.083)	14(14.538)	80(83.333)
		45+ & above	3(10)	6(20)	21(70)
	Literacy	Illiterate	0(0)	2(10.526)	17(89.473)
		Literate	65(30.660)	45(21.226)	152(71.698)
Any physical illness/ abnormalities shown by Thalassaemia carrier	Gender	Male	11(8.396)	20(15.267)	100(76.335)
		Female	4(4)	20(20)	76(76)
	Generation	13-25years	7(6.666)	19(18.095)	79(75.238)
		Up to 45 years	5(5.208)	14(14.583)	77(80.208)
		45+ & above	3(10)	7(23.333)	20(66.666)
	Literacy	Illiterate	0(0)	2(10.526)	17(89.473)
		Literate	15(7.075)	38(17.924)	159(75)
Any mental illness/ abnormalities Thalassaemia carrier	Gender	Male	6(4.580)	18(13.740)	107(81.679)
		Female	1(1)	19(19)	80(80)
	Generation	13-25years	4(3.809)	16(15.238)	85(80.952)
		Up to 45 years	52(54.166)	14(14.583)	30(31.250)
		45+ & above	1(3.333)	7(23.333)	22(73.333)
	Literacy	Illiterate	0(0)	2(10.526)	17(89.473)
		Literate	7(73.301)	35(16.509)	170(80.188)
Any mental disability/ abnormalities among the parents of Thalassaemia patients	Gender	Male	8(6.106)	58(44.274)	65(49.618)
		Female	5(5)	47(47)	48(48)
	Generation	13-25years	8(7.619)	36(34.285)	61(58.095)
		Up to 45 years	4(4.166)	46(47.916)	46(47.916)
		45+ & above	1(3.333)	23(76.666)	6(20)
	Literacy	Illiterate	0(0)	14(73.684)	5(26.315)
		Literate	13(6.123)	91(42.924)	108(50.943)
Thalassaemia was treatable but not curable	Gender	Male	76(58.015)	16(12.213)	39(29.770)
		Female	56(56)	16(16)	28(28)
	Generation	13-25years	58(55.238)	21(20)	26(24.761)
		Up to 45 years	58(60.416(	10(10.416)	28(29.166)
		45+ & above	1653.333)	1(3.333)	13(43.333)
	Literacy	Illiterate	9(47.368)	2(10.526)	8(42.105)
		Literate	123(58.018)	30(14.150)	59(27.830)

Table 6.18: Attitude towards Thalassaemia

Sl.No.	Statements	Strongly Agree	Agree	Disagree	Strongly Disagree
1	A Thalassaemia child was born due to sin committed by parents	164(70.99)	22(9.52)	16(6.92)	29(12.55)
2	I feel uneasy to sit with a person, who was suffering from thalassaemia	138(59.74)	60(25.97)	13(5.62)	20(8.65)
3	The life becomes miserable for entire family, where anyone was suffering from Thalassaemia	55(23.80)	57(24.67)	68(29.43)	51(22.07)
4	The family where someone was suffering from thalassaemia was unlucky	53(22.94)	46(19.91)	75(32.46)	57(24.67)
5	It was better to die than live with a deadly disease like; thalassaemia	39(16.88)	69(29.87)	30(12.98)	43(18.61)
6	I try to avoid a thalassaemia patient	108(46.75)	73(31.60)	24(10.38)	26(11.25)

The above tables show that many respondents strongly Disagreed (70.99 percent) that a thalassaemic child was born due to sin committed by parents, and only 12.55 percent strongly Disagreed on this. Regarding the statement of feeling of uneasiness to sit with Thalassaemia patients, 59.74 percent strongly agreed, 25.97 percent agreed, 5.62 percent Disagreed, and 8.65 percent strongly Disagreed.

Regarding the statement that the life becomes miserable for the entire family, whereas, for anyone suffering from Thalassaemia, 23.80 percent strongly agreed, 24.67percent agreed, 29.43 percent Disagreed, and the rest of 22.07 percent strongly Disagreed. Regarding the statement that a family with a Thalassaemia patient was unlucky, 24.67 percent of respondents strongly Disagreed, 19.91 percent agreed, 32.46 percent Disagreed, and 22.94 percent strongly agreed. Regarding the feeling that it was better to die than live with a deadly diseases like Thalassaemia, 16.88 percent strongly agreed, 29.87 percent agreed, 12.98 percent Disagreed and the rest of 18.61percent strongly Disagreed.

Statement regarding, whether one would try to avoid Thalassaemia patients, most of the respondents (46.75 percent) strongly Disagreed, 31.60 percent agreed; only 10.38 percent Disagreed and the rest 11.25 percent strongly agreed.

Table 6.19: Attitude towards Thalassaemia (item-wise bi-furcation)

Statements	Characteristics	Category	Strongly Agree	Agree	Disagree	Strongly Disagree
A Thalassaemia child was born due to sin committed by parents	Gender	Male	97 (74.045)	13 (9.923)	9 (6.870)	12 (9.160)
		Female	67(67)	9(9)	7(7)	17(17)

	Generation	13-25years	82 (78.095)	4 (3.809)	3 (2.857)	16 (15.238)
		Up to 45 years	64 (66.666)	12 (12.5)	9 (9.375)	11 (11.458)
		45+ & above	18 (6)	6 (20)	4 (13.333)	2 (6.666)
	Literacy	Illiterate	9 (47.368)	3 (15.789)	4 (21.052)	3 (15.789)
		Literate	155 (73.113)	19 (8.962)	12 (5.660)	26 (12.264)
I feel uneasy to sit with a person, who was suffering from Thalassaemia	Gender	Male	78 (59.541)	35 (26.717)	7 (5.343)	11 (8.396)
		Female	60 (60)	25 (25)	6 (6)	9 (9)
	Generation	13-25years	72(68.571)	16 (15.238)	7 (6.666)	10 (9.523)
		Up to 45years	51 (68.571)	36 (34.285)	3 (2.857)	6 (5.714)
		45+ & above	15 (50)	8 (26.666)	3 (10)	4 (13.333)
	Literacy	Illiterate	10 (52.631)	5 (26.315)	0 (0)	4 (21.052)
		Literate	128 (60.377)	55 (25.943)	13 (6.132)	16 (7.547)
The life becomes miserable for entire family, where anyone was suffering from Thalassaemia	Gender	Male	30 (22.9)	35 (26.717)	42 (32.061)	24 (18.32)
		Female	22 (22)	25 (25)	26 (26)	27 (27)
	Generation	13-25years	31 (29.523)	22 (20.952)	26 (24.761)	26 (24.761)
		Up to 45 years	17 (17.708)	27 (28.125)	32 (33.333)	20 (20.833)
		45+ & above	4 (13.333)	11 (36.666)	10 (33.333)	5 (16.666)
	Literacy	Illiterate	4 (21.052)	7(36.842)	4 (21.052)	4 (21.052)
		Literate	48 (22.641)	53 (25)	64 (30.188)	47 (22.169)
The family where someone was suffering from Thalassaemia was unlucky	Gender	Male	28 (21.374)	30 (22.9)	46 (35.114)	27 (20.61)
		Female	25(25)	16 (16)	29 (29)	30 (30)
	Generation	13-25years	32 (30.476)	24 (22.857)	22 (20.952)	27 (25.714)
		Up to 45 years	17 (17.708)	16 (16.666)	39 (40.625)	24 (25)

		45+ & above	4 (13.333)	6 (20)	14 (46.666	6 (20)
	Literacy	Illiterate	5 (26.315)	4 (21.052)	6 (31.578)	4 (21.052)
		Literate	48 (22.641)	42 (19.811)	69 (32.547)	53 (25)
It was better to die than live with a deadly disease like; Thalassaemia	Gender	Male	49 (37.404)	47 (35.877)	16 (12.213)	19 (19.791)
		Female	40 (40)	22 (22)	14 (14)	24 (24)
	Generation	13-25years	46(43.809)	30 (28.571)	11 (10.476)	18 (17.791)
		Up to 45 years	36 (37.5)	29 (30.208)	12 (12.5)	19 (19.791)
		45+ & above	7 (23.333)	10 (33.333)	7 (23.333)	6 (20)
	Literacy	Illiterate	8 (42.105)	7(36.842)	2(10.526)	2 (10.526)
		Literate	81 (38.207)	62 (29.245)	28 (13.207)	41 (19.339)
I try to avoid a Thalassaemia patient	Gender	Male	61 (46.564)	46 (35.114)	13 (9.923)	11 (8.396)
		Female	47 (47)	27 (27)	11 (11)	15 (15)
	Generation	13-25years	54 (51.428)	29 (27.619)	10 (9.523)	12 (11.428)
		Up to 45 years	43 (44.791)	30 (31.25)	10 (10.416)	13 (13.541)
		45+ & above	11 (36.666)	14 (46.666)	4 (13.333)	1 (3.333)
	Literacy	Illiterate	10 (52.631)	6 (31.578)	1 (5.263)	2 (10.526)
		Literate	98 (46.226)	67 (31.603)	23 (10.849)	24 (11.32)

Table 6. 20: Opinion on social distance

Sl.No.	Statements	Very much difficult	Difficult	Little bit difficult	Not at all difficult
1	Living with a person who was suffering from Thalassaemia	150 (64.93)	40 (17.31)	19 (8.22)	22 (9.52)
2	Making friendship with a person who was suffering from Thalassaemia	164 (70.99)	32 (13.85)	12 (5.19)	23 (9.95)
3	Feeling discomfort with a neighbour, who was suffering from Thalassaemia	164 (70.99)	29 (12.55)	18 (7.79)	20 (8.65)
4	Working with a person whose any family member was suffering from Thalassaemia	179 (77.48)	23 (9.95)	8 (3.46)	21 (9.09)

5	Establishing marriage relation in a family where someone was suffering from Thalassaemia	89 (38.52)	68 (29.43)	50 (21.64)	24 (10.38)
6	Accepting a life partner having Thalassaemia trait/carrier	145 (62.77)	10 (4.32)	37 (16.01)	39 (16.88)

The above Table 6.19 shows social distance towards Thalassaemia. When it was asked about living with a person who was suffering from Thalassaemia most of the respondents expressed that it was very difficult (64.93 percent), only 9.52 percent said not at all difficult, 17.31percent said it was difficult, and the rest responded little bit difficult (8.22 percent). About making friendship with a person who was suffering from Thalassaemia most respondents said that it was very difficult (70.99 percent), only 9.95 percent said that it was not at all difficult, 13.85percent said only difficult and the rest 5.19percent responded little bit difficult. About feeling discomfort with a neighbour, who was suffering from Thalassaemia, most of the respondents indicated it as very difficult (82.35 percent), and only 8.65 percent of respondents expressed that it was not at all difficult. 7.79 percent of respondents indicated that it was a little bit difficult and to the rest of respondents (12.55 percent), it was difficult. About working with a person, whose family member was suffering from Thalassaemia, most of the respondents answered that it was very difficult (77.48 percent) and only 9.09 percent said that it was not at all difficult or difficult.

About establishing marriage relations in a family where someone was suffering from Thalassaemia,
Most of the respondents expressed that it was very difficult (38.52 percent), 29.43 percent said that it was difficult, 21.64 percent indicated it was a little bit difficult, and the rest 10.38 percent went for not at all difficult.

For accepting a life partner having Thalassaemia trait/carrier, majority of the respondents that it was very difficult (62.77 percent), 4.32 percent indicated it as difficult; only 16.01 percent and 16.88 percent responded as a little bit difficult and not at all difficult, respectively.

Table 6.21: Social Distance by socio-demographic attributes

Statements	Characteristics	Category	Very difficult	Difficult	Little bit difficult	Not at all difficult
Living with a person who was suffering from Thalassaemia	Gender	Male	84(64.122)	23(17.557)	12(9.16)	12(9.16)
		Female	66(66)	17(17)	79(7)	10(10)
	Generation	13-25years	71(67.619)	14(13.333)	9(8.571)	11(10.476)
		Up to 45 years	60 (62.5)	21(21.875)	7(7.291)	8(8.333)
		45+ & above	19(63.333)	5(16.666)	3(10)	3(10)
	Literacy	Illiterate	16(84.21)	1(5.263)	0(0)	2(94.339)
		Literate	134(63.207)	39(18.396)	19(8.962)	20(9.433)
Making friendship with a person who was suffering from Thalassaemia	Gender	Male	94(71.755)	16(12.213)	8(6.106)	13(9.923)
		Female	70(70)	16(16)	4(4)	10(10)
	Generation	13-25years	78(74.285)	11(10.476)	5(4.761)	11(10.476)
		Up to 45years	66(68.75)	17(17.708)	4(4.166)	9(9.375)

Table 6.22: Social Distance (item-wise bi-furcation)

Statements	Characteristics	Category	Very difficult	Difficult	Little bit difficult	Not at all difficult
Living with a person who was suffering from Thalassaemia	Gender	Male	84(64.122)	23(17.557)	12(9.16)	12(9.16)
		Female	66(66)	17(17)	79(7)	10(10)
	Generation	13-25years	71(67.619)	14(13.333)	9(8.571)	11(10.476)
		Up to 45 years	60 (62.5)	21(21.875)	7(7.291)	8(8.333)
		45+ & above	19(63.333)	5(16.666)	3(10)	3(10)
	Literacy	Illiterate	16(84.21)	1(5.263)	0(0)	2(94.339)
		Literate	134(63.207)	39(18.396)	19(8.962)	20(9.433)
Making friendship with a person who was suffering from Thalassaemia	Gender	Male	94(71.755)	16(12.213)	8(6.106)	13(9.923)
		Female	70(70)	16(16)	4(4)	10(10)
	Generation	13-25years	78(74.285)	11(10.476)	5(4.761)	11(10.476)
		Up to 45years	66(68.75)	17(17.708)	4(4.166)	9(9.375)
		45+ & above	20(66.666)	413.333)	3(10)	3(10)
	Literacy	Illiterate	16(84.21)	1(5.263)	0(0)	2(10.526)
		Literate	148(69.811)	31(14.622)	12(5.66)	21(9.905)
Feeling discomfort with a neighbour, who was suffering from Thalassaemia	Gender	Male	90(68.702)	20(15.267)	12(9.16)	9(6.87)
		Female	74(74)	9(9)	6(6)	11(11)
	Generation	13-25years	75(71.428)	9(8.571)	10(9.523)	11(10.476)
		Up to 45 years	68(70.833)	16(16.666)	6(6.25)	6(6.25)
		45+ & above	21(70)	4(13.333)	2(6.666)	3(10)
	Literacy	Illiterate	17(89.473)	0(0)	0(0)	210.526)
		Literate	147(69.339)	29(13.679)	18(8.49)	18(8.49)
Working with a person whose any family member was suffering from Thalassaemia	Gender	Male	101(77.099)	15(11.45)	5(3.816)	10(7.633)
		Female	78(78)	8(8)	3(3)	11(11)
	Generation	13-25years	81(77.142)	11(10.476)	3(2.857)	10(9.523)
		Up to 45 years	88(91.666)	3(3.125)	1(1.041)	4(4.166)
		45+ & above	2273.333)	3(10)	2(6.666)	3(10)
	Literacy	Illiterate	17(89.473)	0(0)	0(0)	2(10.526)
		Literate	162(76.415)	23(10.849)	8(3.773)	19(8.962)
Establishing marriage relation in a family where someone was suffering from Thalassaemia	Gender	Male	36(27.48)	13(9.923)	36(27.48)	46(5.114)
		Female	32(32)	11(11)	14(14)	43(43)
	Generation	13-25years	37(35.238)	11(10.476)	12 (11.428)	45(42.857)
		Up to 45 years	25(23.809)	9(8.571)	28(26.666)	34(32.38)
		45+ & above	6(20)	4(13.333)	10(33.333)	10(33.333)
	Literacy	Illiterate	6(31.578)	0(0)	5(26.315)	8(42.105)
		Literate	62 (29.245)	24 (11.32)	45 (21.226)	81 (38.207)
Accepting a life partner having Thalassaemia trait/carrier	Gender	Male	25(19.083)	3(2.29)	27(20.61)	76(58.015)
		Female	14(14)	7(7)	10(10)	69(69)
	Generation	13-25years	24(22.857)	7(6.666)	87.619)	66(62.857)
		Up to 45 years	12(12.5)	3(3.125)	21(21.875)	60(62.5)
		45+ & above	3(10)	0(0)	8(7.619)	19(63.333)
	Literacy	Illiterate	0(0)	1(5.263)	5(26.315)	13(68.421)
		Literate	39(18.396)	9(4.245)	32(15.094)	132(62.264)

Table 6.23: Readiness to practice positive attitudes

Sl.No.	Statements	Yes	No
1	Do you try to adopt recommended improved practices in many spheres of life?	213 (92.20)	18 (7.792)
2	Have you ever talked about the benefit of adoption of such improved practices?	195 (84.41)	36 (15.584)
3	Have you ever helped any one to follow such improved practices?	156 (67.53)	75 (32.467)
4	Do you practice what you have learned about diseases?	159 (68.83)	72 (31.168)
5	Are you the first to adopt any improved practices regarding diseases in your village?	25 (10.82)	206 (89.177)
6	Did you ever discuss with your family members about Thalassaemia?	100 (43.29)	131 (56.709)
7	Have you tested your blood for Thalassaemia?	45 (19.48)	186 (80.519)
8	Have you tested blood of your family members for Thalassaemia?	39 (16.883)	192 (83.116)
9	Have you attended any awareness programme related to Thalassaemia?	42 (18.18)	189 (81.818)
10	Do you think that family level counselling was effective to combat these diseases?	211 (91.34)	20 (8.658)

The above Table reveals that among the respondents who are aware of thalassemia, 92.20 percent will tried to adopt recommended improved practices, 84.41percent expressed that they also told others about it, 67.53 percent indicated that they never helped anyone to follow such type of improved practices, and 68.83 percent told that they would practice in their day-to-day life whatever they learn about the diseases.

When it was asked whether they discussed with their family members about Thalassaemia, only 43.29 percent of respondents said ‘yes’ which means discussions regarding any social issues with family members are less in the village. About 19.48 percent of respondents tested their blood for Thalassaemia and 22.07 percent of respondents tested the blood of their family members for it. Although 91.34 percent of respondents think that family-level counselling was effective in combating these diseases, only 18.18 percent of respondents attended any type of awareness programme.

Table 6.24: Awareness about Thalassaemia (Un exposed)

Aspects of TAS	Mean	SD
Knowledge about Thalassaemia	24.286	4.559
Attitude towards Thalassaemia	17.679	4.022
Social Distance Thalassaemia	17.913	4.138
Practice	15.186	1.889

Table 6.25: Gender and Awareness about Thalassaemia

Aspects of TAS	Grouped compared	Mean	SD	t-value
Knowledge about Thalassaemia	Male	24.259	4.782	.051 NS
	Female	24.290	4.279	
Attitude towards Thalassaemia	Male	18.015	3.938	1.444 NS
	Female	17.240	4.107	
Social Distance Thalassaemia	Male	17.985	4.123	.291 NS
	Female	17.820	4.176	
Practice	Male	15.168	1.759	.167 NS

Significant

The above table shows that there was no significant difference between males and females, so far as knowledge, attitude and social distance about Thalassaemia (t-value at p>.05; 0.051, 1.444, 0.291 and 0.167 respectively).

Table 6.26: Generation gap and Awareness about Thalassaemia

Aspects of TAS	Grouped c	Compared Mean	SD	F-value
Knowledge about Thalassaemia	13-25 years	25.286	5.271	2.003 NS
	26-45 years	23.594	4.313	
	46 & above	23.667	5.352	
Attitude towards Thalassaemia	13-25 years	18.190	4.067	3.400*
	26-45 years	17.344	3.803	
	46 & above	16.967	4.445	
Social Distance Thalassaemia	13-25 years	18.105	4.339	1.866 NS
	26-45 years	17.875	3.820	
	46 & above	17.367	4.476	
Practice	13-25 years	15.743	1.781	1.302 NS
	26-45 years	14.760	1.739	
	46 & above	14.600	2.222	

* Significant at .05 level

The above Table revealed that irrespective of sex and education, significant difference was revealed among the three groups, so far as knowledge, attitude, social distance and practice are concerned (F= 2.003, 3.004, 1.866 and 1.302, respectively). It indicates that with that age was the only causative factor on knowledge aspect, but it was seen from mean values that with the increase of age, attitude changed in positive direction, which ultimately helped to minimize the social distance for different social issues.

Table 6.27: Education and Awareness about Thalassaemia

Aspects of TAS	Grouped compared	Mean	SD	t-value
Knowledge about Thalassaemia	Illiterate	21.526	4.047	3.093**
	Literate	24.533	4.529	
Attitude towards Thalassaemia	Illiterate	17.526	5.264	.092 NS
	Literate	17.693	3.907	
Social Distance Thalassaemia	Illiterate	18.211	4.626	.289 NS
	Literate	17.888	4.103	
Practice	Illiterate	14.474	2.169	1.500 NS
	Literate	15.250	1.855	

* *Significant at .01 level; NS= Not Significant.

The above table shows that education has a good impact regarding knowledge about Thalassaemia. But it might not be a significant causative factor for positive attitude towards Thalassaemia and social distance about it. In this regards it can be presumed that proper family counselling was very much essential for improvement of attitude towards Thalassaemia and to minimize the social distance between the thalassaemic traits and normal person.

Table 6.28: Recommendation regarding Awareness about Thalassaemia

Sl.No	Statements	Answer		
		Very much effective	Much effective	Less Effective
1-	How much effective would be the community level awareness programme to combat this problem?	174 (75.32)	55 (23.80)	2 (.865)
2-	If yes, how it can be organized? a-at local level (Panchayat) b-at area level (Block) c-at bigger network level/city level (District) d-at state level e-at national level	199(86.14) 7(3.03) 3(1.29) 4(1.73) 18(7.79)		
3-	Do you think that any awareness programme at the national level was more effective?	Yes 102 (44.15)	No 64 (27.70)	Can't say 65 (28.13)
4-	Do you think that any person can contribute something to the solution of this problem?	Yes 140 (60.60)	No 38 (16.45)	Can't say 5322.94)
5-	What will be your role? i. Convey message to others about it ii. Blood should be tested before marriage iii. Participation in such type of awareness programme iv. No comments			

It was observed from above Table that a community-level program would be effective in combatting these diseases because 75.32 percent of respondents said it would be very effective, and 23.80 percent indicated much more effective; only .865percent expressed that it would be less effective. Majority of respondents (86.14 percent) indicated that awareness program could be organized at local level, whereas 7.79 percent expressed at national level, 3.03 percent told block level and only 1.73 percent and 1.29 percent expressed at state level and district level.

Though most of the respondents said that awareness program can be organized at the local level; 44.59 percent thought that any awareness program at the national level would be more effective. When it was asked whether any person could contribute something to the solution of this problem, 60.60 percent answered 'Yes'.

Comparisons between the Level of Awareness of Exposed Villages and Relatively Unexposed Villages

The present section tries to compare the results to see whether the two groups differ significantly or not among themselves on the various measures of TAS. To test the mean differences on the various measures of TAS scores obtained from the different sub-groups, t-value and F-test were applied as and when required.

Table 6.29: Level of Awareness about Thalassaemia

Aspects of TAS	Grouped compared	Mean	SD	t-value
Knowledge about Thalassaemia	Exposed	26.564	5.606	5.916**
	Unexposed	24.286	4.559	
Attitude towards Thalassaemia	Exposed	15.161	3.032	8.535**
	Unexposed	17.679	4.022	
Social Distance Thalassaemia	Exposed	14.769	3.007	10.445**
	Unexposed	17.913	4.138	
Practice	Exposed	14.373	2.197	5.211**
	Unexposed	15.186	1.889	

** Significant at .01 level.

The above table shows the comparison of mean scores of various measures of TAS. All the t-values as reflected in the table revealed a significant difference between respondents from exposed and relatively unexposed villages. The finding indicates that awareness tends to be related to the social amenities available in the village. In other words, people from exposed villages seemed to be more aware than people belonging to that village which has no such exposure.

Table 6.30: Gender and Awareness about Thalassaemia

Aspects of TAS	Grouped compared		Mean	SD	t-value
Knowledge about Thalassaemia	Male	Exposed	26.354	2.828	4.665**
		Unexposed	24.259	4.782	
	Female	Exposed	25.805	1.414	3.466**
		Unexposed	24.290	4.279	
Attitude towards Thalassaemia	Male	Exposed	15.151	2.828	7.517**
		Unexposed	18.015	3.938	
	Female	Exposed	15.175	2.756	4.619**
		Unexposed	17.240	4.107	
Social Distance Thalassaemia	Male	Exposed	14.621	3.021	8.431**
		Unexposed	17.985	4.123	
	Female	Exposed	14.952	2.621	6.666**
		Unexposed	17.820	4.176	
Practice	Male	Exposed	14.395	2.128	3.923**
		Unexposed	15.168	1.759	
	Female	Exposed	14.347	1.414	3.835**
		Unexposed	15.210	2.056	

** Significant at .01 level.

The above table compares the mean scores of various combinations of male and female respondents on various measures of TAS. The data presented in this table reveals that respondents from exposed villages as well as respondents from relatively unexposed villages' male and female groups differed significantly on all the aspects.

Table 6.31: Generation gap and Awareness about Thalassaemia

Aspects of TAS	Grouped compared		Mean	SD	t-value
Knowledge about Thalassaemia	13-25 years	Exposed	24.761	2.828	.945NS
		Unexposed	25.286	5.271	
	Up to 45 years	Exposed	26.080	4.949	4.646**
		Unexposed	23.594	4.313	
	Above 45 years	Exposed	25.620	1.823	1.96*
		Unexposed	23.667	5.352	
Attitude towards Thalassaemia	13-25 years	Exposed	15.316	3.435	6.101**
		Unexposed	18.190	4.067	
	Up to 45 years	Exposed	15.166	1.414	5.486**
		Unexposed	17.344	3.803	
	Above 45 years	Exposed	14.491	1.414	3.001**
		Unexposed	16.967	4.445	
Social Distance Thalassaemia	13-25 years	Exposed	15.027	5.078	5.438**
		Unexposed	18.105	4.339	
	Up to 45 years	Exposed	14.825	1.706	7.595**
		Unexposed	17.875	3.820	
	Above 45 years	Exposed	14.569	1.707	3.342**
		Unexposed	17.367	4.476	
Practice	13-25 years	Exposed	14.508	1.054	6.493**
		Unexposed	15.743	1.781	
	Up to 45 years	Exposed	14.264	1.717	2.407**
		Unexposed	14.760	1.739	
	Above 45 years	Exposed	14.425	2.121	.376NS
		Unexposed	14.600	2.222	

* Significant at .05 level; ** Significant at .01 level; NS Not significant.

It was evident from t-values for all the sub-groups on various measures TAS turned out to be significant except on practice adopted for Thalassaemia of 26.45 years age group and more than 45 years age group. It indicates that exposure of the village plays significant role to gain the knowledge about any social issues.

Conclusions, Implications and Recommendations

General Findings

It was observed from demographic characteristics of the sample (exposed villages and relatively unexposed villages) that the numbers of female respondents were less than male respondents. Most of the participants reported having studied up to secondary level. (Exposed villages 42.52 percent and relatively unexposed villages 44.20 percent). Most of the respondents were within the age range of 26-45years. (Exposed villages 52.83 percent and relatively unexposed villages 51.20 percent). Most of the respondents were married (Exposed villages 79.53 percent and relatively unexposed villages 75.76 percent) and belonging to nuclear family. The occupation pattern of the respondents was mixed like-agricultural labour, daily wage labour, business, service etc. Most respondents belonged to Scheduled Caste in both the exposed and unexposed categories of villages.

Most respondents (79.78 percent) had seen/known a child whom blood has given frequently but only 12.229 percent told that it was transmitted by parents in case of exposed villages,

whereas samples from relatively unexposed villages 61.835 percent had seen/known a child whom blood has given frequently and only 15.458 percent responded that it was transmitted by parents. Regarding knowledge about genetic diseases the responses were same in both exposed (41 percent) and unexposed (39.612 percent) villages. Their responses were- asthma, TB, cancer, blood sugar, Thalassaemia, AIDs etc.

It was observed from comparison between the sample groups viz., exposed and relatively unexposed that 57.39 percent of the participants from exposed villages reported to have had no knowledge about Thalassaemia; even they never heard the word 'Thalassaemia', whereas respondents from unexposed villages 81.40 percent never heard the word 'Thalassaemia'.

Level of Awareness status of Thalassemia

Altogether 2493 persons above 13 years (1251 from exposed villages and 1242 from unexposed villages) were contacted; of which 764 (533 from exposed villages and 231 from unexposed villages) persons were found to have heard the word 'Thalassaemia' and were included for further extended interview with a pre-structured six sub-scale schedule. The percentage of respondents was more in exposed village than relatively unexposed village. The 226 (42.40 percent) respondents from exposed villages were known about the fact that it was an inherited disorder and among them, almost all i.e. 210 (39.399 percent) also knew that it was a blood disorder. But surprisingly opposite picture came in case of the respondents from unexposed villages. The 83.11percent respondents knew that it was a disorder of blood but only 53.24 percent told that Thalassaemia was an inherited disease. It might be just because the thalassaemia patients were there in all the four selected unexposed villages. About 104 (19.512 percent) respondents knew that β-Thalassaemia trait was a type of Thalassaemia, whereas only 50 (9.380 percent) could identify that there are other types of Thalassaemia, although only 19 (3.564 percent) could exactly name them. But in case of the respondents from unexposed villages only 13.41percent (31) had knowledge about β-Thalassaemia trait and only 4 (1.73 percent) could name them.

About 95 percent of respondents from exposed villages and 90 percent respondents from unexposed villages opined that getting married to a family with β-Thalassaemia major child was difficult and not a good preference. The view that the presence of a β-Thalassaemia major child brings in economic and social havoc to the family was commonly opinionated by about 59 percent of the respondents. However, 44 percent did not think that a β-Thalassaemia major child was to be treated as a burden on the parents in exposed villages. In case of respondents from relatively unexposed villages it was same (48.47 percent).
20.075 percent of respondents opted for getting tested for β-Thalassaemia in exposed villages, but percent of respondents opted for getting tested for β Thalassaemia has dropped in relatively unexposed villages (19.48 percent).

Suggestions

Majority of respondents (78.236 percent from exposed village and 92.20 percent from relatively unexposed village) felt that if improved practice (mass awareness and availability of detection facilities for β Thalassaemia) can be made available, it will be beneficial to the general people. Majority of respondents (92.495 percent from exposed village and 99.12 percent from relatively unexposed village) felt that a community-based program would be of more effective than a nation-wide prevention program.

Chapter 07

Conclusion

Haemoglobinopathies are hereditary disorders caused by point mutation. There are over 750 haemoglobin variants across the globe but in India the common variants are sickle cell anaemia (SCA), thalassaemia (Hbβ-thal and Hbα-thal), HbD, HbE, are. They pose a major health problem in India. With an ethnically diverse population of over 144 million, India had reported to have over 3.5 million carriers. Globally, thalassaemia was more prevalent than the sickle cell anaemia, but in India the magnitude and burden of sickle cell anaemia was much higher than thalassaemia. Sickle cell anaemia was qualitative in nature whereas thalassaemia was quantitative one since the Hbβ-thal gene doesn't allow the globin moiety of the molecule to form the blood in human body.

The present study revealed a significant regional difference in the occurrence of haemoglobinopathies. Table 7.1 summarizes the prevalence of major haemoglobinopathy in the Andaman Islands, Central India, Eastern India, and the North-eastern India. But earlier studies show high prevalence rate of HbS in southern states. There were rare cases of compound heterozygotes of sickle cell and β-thalassaemia interacting with HbE in the present study, despite high frequencies of HbS and β thalassaemia as these are clinically severe and are identified at an early age. The survey mainly targeted premarital age group for early detection of the gene to prevent from transmitting it from one generation to the next.

Table 7.1: Region-wise prevalence of haemoglobinopathies

Regions	Abnormalities detected		
	HbAS	HbBTT	HbAE
Andaman Islands	1.62	5.09	3.24
Central	7.61	1.98	0
Eastern	0.04	6.16	3.17
Northeastern	0	0	22.89

From the region wise distribution in table 7.1 it is observed that due to the intermixing of gene in the past, the Andaman Islands exhibits combination of three genes-HbS, β-thal and HbE which attributes to the open marriage system. In the central region, occurrence of sickle cell gene is considerably high with sporadic incidences of β-thal despite high concentration of Sindhi population in the region but free from HbE gene. Ironically, in the late fifties and early sixties the magnitude of HbS gene was alarming in central India (Shukla and Solanki, 1958; Das et. al.1961) but the present study shows moderate to high frequency of HbS gene in tribal, lower castes, and higher castes with complete absence in some of the communities.

The tribal people of central and southern had a geographical unicentric origin and had unicentric origin of the mutated gene when these tribal populations were in direct contact and underwent panmixia or gene flow. But now they dispersed and live distantly isolating themselves and maintain strict endogamy leading to high frequency for HbS gene. A high frequency of SCT was reported among the food gatherers and jhoom/slash burn agriculturist in India (Harrison, 1988). But the distribution pattern of HbS does not conform to the earlier studies as the HbS in high frequencies is prevalent among the tribes with varied lifestyles. For example, the Pardhan, the Gowari (Central India) are agriculturist; the Irula (Tamil Nadu) is an artisan; the Kurumba (Karnataka), the Madia (Central India), are hunting tribes and the Banjara (Central India) is a nomad, show equally high frequencies of HbS amongst them. On the contrary, the Mana is an agriculturist, the Halba, weaver (Central India), and the Khutia Khond

(Odisha) are hunters and gatherers show a complete absence or negligible HbS gene. Therefore, sickle cell anaemia was more prevalent in southern, central, western and some part of eastern India. Contrarily, thalassaemia was found to be preponderance among the Sindhi population of different geographical areas and Bengali population in eastern India. Since thalassaemia was a Mediterranean in origin, the human migration took place prominently through the sea route to the western and the eastern parts of India, hence the high occurrence of in these two regions of India. Prevalence of thalassaemia and HbE in the eastern region was considerably high with scarce sickle cell gene. In the north-eastern part of the country HbE was reported to be alarmingly high reaching to about 22.89 per cent may be due to stringent rule of endogamy in tribal populations. Tibeto-Burman, Mon-Khmer and Austro-Asiatic linguistic populations share genetic commonalities. The occurrence of larger frequency of HbE in populations of Northeast India, Laos, Thailand and Cambodia forms 'haemoglobin E quadrangle'.

IVS-1-5 (G>C) which is an Asian Indian mutation, is the commonest mutation in the eastern region. Studies shows that majority of the people from Gujarat, Uttar Pradesh and Maharashtra also have this mutation.

A community-based approach to control the birth of clinically severe children was of immediate need during the period of study and the study significantly created awareness in the regions. However, a definite lack of awareness and acceptance to medical interventions during pregnancies among the general rural populations seems to be other most important factors behind the less response to the preventions of haemoglobinopathies. Therefore, more attention was given towards the premarital screening and counselling which addressed to suggestions of avoiding marriages between two carriers of haemoglobinopathies, which seems to be conventional in the studied areas. The response depended on the level of interaction and the magnitude of education imparted. The approach indicated that it was not impossible to generate adequate awareness among a cross section of mass populations which have led to a successful prevention of haemoglobinopathies at community level. About 57percent of all respondents attending the haemoglobinopathies screening camps who previously had no idea about the differences between thalassaemia trait and diseases, reported the source of the idea they got from was the awareness drive and camps organized by the Anthropological Survey of India. Moreover, additional eagerness to know about thalassaemia was found especially among the women and younger generations. It was pertinent here to mention that two demographic variables, marital status and religion, did not seem to be significantly effective on the thalassaemia awareness scale and its other sub-scales. The approach undertaken by Anthropological Survey of India assures to be a benchmark for future reference and intervention programs in controlling haemoglobinopathies in Indian communities.

To summarise, the study:

1) Identified groups at high risk through mass screening at the community level. and screening of pre- marital/reproductive age individuals.

2) Informed carrier couples, detected positive for haemoglobinopathies, were at risk genetically and councelled for prenatal diagnosis.

3) Led to a significant reduction in affected births with the awareness, mass screening and counselling.

4) Laid down strategy for prevention-provision of prenatal diagnosis for risk couple was initiated.

5) Included protection of cultural, religious and ethical aspects during Genetic counselling.

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Appendix

Consent Form

ANTHROPOLOGICAL SURVEY OF INDIA
Ministry of Culture, Department of Culture
Government of India
27, Jawaharlal Nehru Road, Kolkata–700 016
Dial: 91 33 2286 1781/1733, Fax: 91 33 2286 1799
E-mail: dnalabhq@ansi.gov.in Website: www.ansi.gov.in

Project Name: ***Molecular Characterization of Haemoglobinopathies and beta-Thalassaemia in West Bengal***

Consent Form

1. I am giving blood sample with my own wish knowing fully well about the purpose of the collection of blood sample.

2. I consent to the test (s), which I understand will be based on DNA.

3. I agree to the request of Anthropological Survey of India (AnSI) to use my blood sample for genetics studies, which may lead to the discovery of new techniques or improvising the existing one. Furthermore, I also allow investigators at AnSI to use my blood sample for research purposes that may facilitate better understanding of the human genome, provided confidentiality of the identity of the sample is maintained.

4. I also allow investigators to publish the data obtained from the aforementioned studies.

Name of the Subject (in Capital) :

Signature with date and address of the parent/guardian of the subject is under 18 years of age :

Signature of the Subject with date and address :