GOVERNMENT OF THE PEOPLE'S REPUBLIC OF BANGLADESH
Ministry of Health and Family Welfare
Non Communicable Diseases Control Programme,
Directorate General of Health Service (DGHS)
Mohakhali, Dhaka-1212

Final Report

Survey for Determination of Prevalence of Thalassaemia and Launching of Economic Screening Tool to Adopt in National Health Policy to Prevent Thalassaemia in Bangladesh.

June 2016

BTS-CRDS Joint Venture

Green Garden Tower, Level-6, 25/A, 25/B, Biruttam KM Shafiullah Sarak, Green Road, Dhaka-1205.
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Executive Summary

Background: Bangladesh is a high prevalence country in consideration of Haemoglobin disorders. It is considered that among its 170 million people 4.1% (range 1-5%) are carriers of beta Thalassaemia, a non communicable disease, while 6.1% (range 4-10%) are carriers of the variant HbE. This means that an estimated 14500 new births of affected children are expected every year. A current estimate is that there may be up to 90000 patients with clinically significant Haemoglobin disorders living in the country at any time. The purpose of the study is to know about the Thalassaemia burden in Bangladesh and to develop a tool to diagnose Thalassaemia based on patients economic characteristics.

The Non-communicable Diseases include cardiovascular disease, diabetes, chronic respiratory diseases and certain cancers have become a global problem accounting for more than 68% of the total global deaths. NCD result in significant socio economic and health care costs and is detrimental to sustainable development. The chronic nature of the diseases requires protracted treatment and can lead to catastrophic expenditure particularly among the poor. One of these is Heamoglobinopathies commonly referred to as Thalassaemia.

Thalassaemia/Haemonoglobinopathy is a genetic disorder affecting the blood cells. It is characterized by decline, below the natural rate, in both the red blood cells and the level of Haemoglobin. In world now almost 7% of the total world populationhas Haemoglobin disorders, 300,000 – 500,000 children are born with Haemoglobin disorders, 70% of children are born with sickle cell anemia, and the rest are living with Thalassaemia. Every year, 50-80% of children die from the sickle cell anemia. Every year, 50,000 – 100,000 children die from major Thalassaemia over the globe.

Thalassaemia is the name of a group of genetically inheritedHaemoglobin disorder passed down through families in which the body makes an abnormal form of Haemoglobin, the protein in red blood cells that carries oxygen. It results in excessive destruction of red blood cells, which leads to anemia. Patients have a lower-than-normal number of red blood cells in their bodies and too little Haemoglobin.

Understanding: The government in its Vision 2021 *the government seeks to create conditions whereby the people of Bangladesh have the opportunity to reach and maintain the highest level of attainable health.* The 7th Five year goals includes meeting challenges of emerging and re-emerging and non-communicable diseases, health hazards due to climate change and emergency response to catastrophe. Haemoglobinopathy being a non communicable disease having immense impact on the society needs to be addressed properly. Therefore a national program should include a policy of prevention with a view to limiting new births with the disease. The proposed research will help in understanding the problem with greater clarity and for the development of a National Plan to contain Thalassaemia (Haemoglobinopathies) in Bangladesh and adopting an economic screening tool.

The objectives of assignment are to obtain information addressing the following specific parameters:

- Estimate Thalassaemia prevalence in Bangladesh
- Describe the characteristics of the patients.
- Develop an economic screening tool to diagnose Thalassaemia in Bangladesh.

In view of attaining the objective the following scope of works has been set in the Terms of Reference (TOR).

Review of literature

Review existing literature available globally and nationally on Thalassaemia.

Develop a tool which may diagnose Thalassaemia based on economic background of a probable patient

Complete the assessment and submit a report

The rate prevalence of Thalassaemia/Haemoglobinopathy in Bangladesh is not well documented. A study on Bangladeshis living in UK is cited as a document by World Health Organization. The preliminary survey by Bangladesh Thalassaemia Samity (BTS) on 1800 samples depicts that the Thalassaemia and Hb–E carriers is around 10%. The carriers are divided between β Thalassaemia and Hb-E Haemoglobinopathy.

Different techniques like Thalachip technique, Complete Blood Count (CBC), Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT), Capillary Electrophoresis (CE); etc has been used to diagnose Thalassaemia and other Haemoglobinopathies. Sensitivity, Specificity and the Accuracy, positive predictive value and Negative value vary from method to method. But International Thalassaemia Foundation recommends Capillary Electrophoresis (CE) for diagnosis.

The scope of study requires intense literary review and analysis. Support for field activity is ensured in the ToR. In the proposal, in consideration of obtaining some primary data has been proposed as a part on a pilot scale under the study.

Approach and methodology: It included tasks related to the attainment of the objectives. The tasks carried out included collection, review and analyses of local and global literature, development of data collection instruments, sample design for testing blood sample and collecting opinion from volunteers, development of field survey plan for primary data, collection of blood samples and household data, testing of blood samples, collection of secondary data, preparation of data base from secondary and secondary sources in MS Excel and analyses of all data for report preparation. In addition some FGDs and KKIs were carried out to gather opinions of the community and Specialists respectively.

Prevalence**:** The test results of 1439 samples from university college students mainly at Dhaka, Bogra and Noakhali depict that in Bangladesh 10.2% Hb E carrier, 3.7% beta Thalassaemia carrier and about 1% Hb E diseased who do not face problems except under certain conditions. The male female distribution shows 14. 7% for females while 13.6% for males.

In Barisal Division in all 10.4% persons were either carriers of Thalassaemia or diseased. Among the carriers 5.9% were carriers of Hb E Haemoglobinopathy while 2.9% were carriers of B Thalassaemia and 2.9% were Hb E diseased.

In Chittagong Division, in all 12.3 % persons were carriers of either form of Thalassaemia prevalent in the country. The Hb E carriers were 8.1 % and others 4.1 %. There is indication that the hill districts have high rate of occurrence.

In Dhaka Division the overall occurrences of Hb E and βThalassaemia carriers are 8.2% and 2.5% respectively. The percentage of Hb E Disease is 0.8 %.

In Khulna Division the overall occurrences of Hb E and β Thalassaemia carriers are 5.0% and 1.3% respectively.

In Rajshahi Division the overall occurrences of Hb E and βThalassaemia carriers are 13.0 % and 2.0% respectively. The percentage of Hb E Disease is 0.1 %. The occurrence Hb E in Rajshahi District is more than 27%

In Rangpur Division the overall occurrences of Hb E and βThalassaemia carriers are 26.7% and 4.6% respectively. The percentage of Hb E Disease is 3.0 %. In Dinajpur District 47.8 % of the people are bearers of Haemoglobinopathy traits

In Sylhet Division the overall occurrences of Hb E and βThalassaemia carriers are 7.1% and 7.1% respectively. The male female distribution shows 12. 8% for females while 15.6% for males.

Patient Characteristics: In all 215 patients/patients guardians were interviewed and record checked. It was found that the occurrence E β Thalassaemia is 49.7% followed by β Thalassaemia by 49.8%. The occurrence of F/β Thalassaemia is only 0.5%. Almost equal number of patients come from both the traits but the number of Hb E trait bearers is almost three times than that of the β trait bearers.

Most of patients (31.6%) were detected of the disease before the age of six months. Next comes the patients (23.1%) in the age group more than 36 months. 4.7 % patients could not tell the age of first detection.

The most common method for diagnosing affected patients was elctrophoresis. This method is available in only some selected places mostly in Dhaka and one or two other divisional towns. CBC was used in only 5.1% cases, but it gave indicative values for which almost all had to come for electrophorasis for confirmation.

Different symptoms were identified by the guardians of the patients before coming for treatment. Loss of appetite was reported by 61.4% followed by enlarged spleen (51.6%) and improper body growth (48.8%). Jaundice, pale face and Hepatitis, which are almost synonymous was felt by 68.4% of patient's guardians. 1% could not give any response.

Among the guardians of sample patients only 27.9% (31 parents) considered that the operation of spleen to be problematic. Among the patients who underwent spleen operation, 87.1% was taking penicillin prophylactic medication.

In the country 61.2% of patients are required to transfuse blood 12 times a year or more. 28.5% patients need transfusion 6 – 12 times a year whereas only 10.3% need transfusion less than six times a year.

About 97.6% of patients used packed cell. Among the types washed packed cell, packed cell and packed cell with filter are used by 63.6%, 31.1 and 2.9% patients respectively.

Among the patients having blood transfusion 91.4% informed that iron chelation medicines are taken by them. Most (70.2%) of the patients used Capsule Kelfer (Deferiprone). No medicine is taken by 3.7% of the patients.

About 72.3 % of patients using medicines gave their opinion in respect of side effects. Among them 10.9% said that no side effect was felt by them. Loss of appetite is most commonly felt by 69.9% of the patients followed by pain at joint by 48.7%. The other problems faced are allergy (14.2%) and respiratory problems (7.1%).

The most common problem stated by 45.1 percent of the households are pecuniary. Non availability of treatment facility is reported by 43.7% of patient followed by non availability of safe blood by 39.1 % households. Lack of diagnostic facility was expressed by 16.7% households. No response and others accounted for 3.7% households.

About 45.3% of the households are from the poor having monthly income of less ran Tk. 10000 per month, while 34.9% are from lower middle class having income in the range of Tk 10,000 – 25,000 and remaining 19.8%are in the from upper middle class and above.

Multiple problems were stated by the patients in their day to day life. Intrinsic problems related with health were faced by 22% while social ones were faced by remaining 78 %

***Awareness*:** A pilot survey among 537 households with college and university students was carried out with responses from the students. It is found that 51.2% did not know what type of disease it is. 39.3% knew that it was a non-contagious disease (NCD). About 8.9% of the respondents thought it to be contagious and 0.6 % considered it to be psychological disorder.

Among those (39.3%), who considered it as NCD 6.16 % could not tell what type of type it was. Among them 4.27 % this considered to be another type of disease. However, 89.6 % of them could tell that it was genetic disease. Thus among all respondents only 35.2%, 2.4% and 1.7% respectively considered it as genetic, infectious and other type of disease.

The source of knowledge of 34.1% household members was family and friends followed by social media (25.1%). The print media accounts for the awareness of about one fifth of the persons. Knowledge from medical professionals is only 2.4%.

The discussion with 215 patient guardians/ Patients reveals that more than 85% of the patient households understood that Thalassaemia is a non contagious genetic disease. They rather have a clear idea about the disease. Another 6.5% understands that it is non contagious but its genetic role is not understood. About 0.5 % of the households still consider it to be contagious disease. About 8% of these households consider this to be physical or other type of problems.

In spite of the close interaction with hospital and doctors only 63.7 % of the guardians know that it occurs only when both the parents are Thalassaemia trait bearers. About 7 % consider that occurs like other diseases and have no relevance with the Thalassaemaic blood characteristic of the parents. About one third (29.3%) households have no clear idea how the disease occurs.

About 94.4% of patient households understood the problem. They informed that different symptoms occur and fever is the most common of these.

The community as a whole is not aware of the problem. But once appraised they gave suggestions for facing the problem.

The discussion with 215 patient guardians/ Patients reveals that more than 85% of the patient households understood that Thalassaemia is a non contagious genetic disease. They rather have a clear idea about the disease. Another 6.5% understands that it is non contagious but its genetic role is not

Findings of Review: Bangladesh with a current population of about 170 million and a carrier percentage of 10.2 (about 6.1% Hb E and 4.1% Beta-Thalassaemia carriers) has an estimated birth of about 14,500 patients every year. That means in any year 14,500 patients are being born and 14,500 patients who were born 6 years ago are moving towards death. That means, about 90,000 families are suffering.

Thalassaemia is a major health problem in Bahrain, the Islamic Republic of Iran and Saudi Arabia, China, India, Indonesia, Malaysia, Singapore and Thailand. The governments in these countries have adopted policies in this regard and effects are stated below.

Sri Lanka: National Thalassaemia prevention program adopted a policy of “safe marriage” that is defined as a marriage where at least one of the partners in a couple is not a carrier for Thalassaemia. I**ran:** Thalassaemia preventio*n in Iran was initiated in 1995. It included* carrier couple screening and genetic counseling to at-risk couples are practiced. The prevalence of β-thalassaemia births in Southern Iran decreased by 81.1%The incidence of β-thalassaemia decreased by 96.5% over 10 years in C*entral Iran*. Iran's successful β-thalassaemia reduction placed the Iranian PMSGC (pre marital screening and genetic counseling) programme as a benchmark for other nationalprograms. **Iraqi Kurdistan:** The PMSGC programme was mandated in 2008 in Kurdistan, Northern Iraq, with Pre-natal diagnosis (PND)

and therapeutic abortion available. The number of thalassaemia affected births in Kurdistan decreased by 65%. **Bahrain*:*** The PMSGC programme was mandated in 2004, and PND and therapeutic abortion are legal. A cross-sectional study indicated a 43.3% at-risk marriage cancellation rate. ***Jordan:*** The PMSGC programme was mandated in Jordan in 2004. S**audi Arabia:** The national PMSGC programme, mandated in 2004, offers screening to all couples registered to marry. The cancellation of at-risk marriage increased from 9.2% in 2004 to 51.9% in 2009 was found. **Turkey:** PMSGC was mandated in Turkey in the 1990s (in Denizli from 1995 and in Mersin from 1998). PND and therapeutic abortion are legal. A 4-year evaluation in Denizli found a 13.3% marriage cancellation rate among at-risk couples while 40% sought PND, with 1 foetus diagnosed with β-thalassaemia and terminated. **Greece:** Greece started their prevention program in 1975and after 35 years the reports indicate significant decrease in Thalassaemia births.

Policy Recommended for Bangladesh: In most of the countries where Thalassaemia disease is a major public health the following are concerns to be considered::

- National programmes for its control do not exist.
- Basic facilities to manage patients are absent,
- Screening for thalassaemia disease is not a common practice
- The diagnosis of the disease is made when severe complication occurs.

Specific Recommendation on tools: Though NESTROFT and DCIP are less expensive tools for screening Beta thalassaemia and Hb E traits; however they are prone to be erroneous. CBC screens iron deficiency anemia or anemia due to some genetic heamoglobin disorder. Hb electrophoresis by Capilary electrophoresis (CE) or HPLC detect the genetic heamoglobin disorder trait as well as disease with confirmation.

NESTROFT + DCIP + Hb electrophoresis will be very cumbersome process to control where as CBC will not identify Hb-E.

Under the circumstances the study suggests to use Hb electrophoresis as a main tool for mass screening.

In our opinion, a carrier detection procedure should be designed to avoid missing any couple at risk. For this reason we have included in the first group of examination, in addition to determination of MCV–MCH, the quantitative evaluation of HbA_2, which may be obtained by electrophoresis or by high-pressure liquid chromatography (HPLC). HPLC has the additional advantage to quantitate also HbF and to detect clinical relevant Hb variants including Hb Knossos (a mild β-thalassemia allele), HbS, HbC, HbD Punjab, HbO Arab and HbE, all of which may interact with β-thalassemia heterozygosity leading to thalassemia major or intermedia or sickle cell β-thalassemia

The following are General recommendations on policy:

1. A broad based screening is to be undertaken through out the country based on scientific statistical sampling method to identify the prevalence trait bearers. (Approximately a sample size of around 25,000 may suffice)
2. Premarital Screening Programme: All people getting married should be screened before their marriage is solemnized. Accordly a law for Safe Marriage is to be enacted.
3. Setting up Thalassaemial screening and genetic counseling programmes: The disease should be identified during the prenatal period or at birth as part of a routine screening programme. Genetic counseling and screening can lead to reduction in the number of children born with the trait. The programme should be developed at the primary care level with appropriate referral system.
4. Parents must be counseled to seek medical care for all febrile events in children with Thalassaemia diseases.

5. Training of health personnel in prevention, diagnosis and case management should be an integral part of the national programme.
6. Integration of national control program for Thalassaemia disease within the national programmes for prevention & control of non communicable disease (like Cancer, Diabetes).
7. Setting up the antenatal screening programm: Antenatal tests shall be carried out on pregnant women, who are known carriers and whose spouses are also carrier for the trait, subject to approval having been obtained from the pregnant women and their spouses.
8. Setting up prenatal screening programme: To Identify women/couples at risk of a pregnancy with thalassaemia disorders and provide appropriate referral & care for prenatal diagnosis with continuation or termination of pregnancy according to family's choices.
9. Vigorous awareness campaign (like family planning, immunization, etc.) on the prognosis of thalassaemia disease through print media, mobile network, electronic media and through distribution of leaflet, booklets poeters, etc is to be carried out,
10. Inclusion of information on Heamoglobanopathies in school curriculum for continuous dessimination among students.
11. Preparation of central digital Thalassaemia patient database and issue identity card to ensure privilege of getting treatment support in public and private health centers.

ABBREVIATIONS

Symbol	Abbreviations
α	Alpha
β	Beta
γ	Gamma
δ	Delta
ε	Epsilon
θ	Theta
%	Percentage
µ	micro
µg	micro-gram
µl	Micro-Liter
^{0}C	Degree Celsius
CBC	Complete Blood Count
CBC	Complete Blood Count
CE	Capillary Electrophoresis
CVS	Chorionic villus sampling
DCIP	Dichlorophenol Iodophenol Precipitation
DFO	Deferoxamine
DFP	Deferiprone
DFX	Deferasirox
FBS	Foetal Blood Sampling
gm	Gram
Hb A	Adult Hemoglobin
Hb E trait	Hemoglobin E trait
HbA_2	Hemoglobin A2
HbF	hemoglobin F (Fetal hemoglobin)
HbS	hemoglobin S (Sickle hemoglobin)
HCT	Hematocrite
HgB	Hemoglobin
HPLC	High Performance Liquid Chromatography
Kg	Kilogram
M	Male
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin concentration
MCV	Mean Corpuscular Volume
ml	Milliliter
Neg	Negative
NESTROFT	Necked Eye Single Tube Osmotic Fragility Test
NR	Non Reactive
NS	Not seen
R.B.C.	Red blood cells
SCT	Sickle cell thalassemia
W.B.C.	White blood Cells

Key Words:

Thalasaemia, Heamoglobinopathy, genetic counseling, pre natal diagnosis, screening, antenatal diagnosis, treatment, homozygote, heterozygote, CBC, NESTROFT, HPLC, CE , Blood Transfusion, Iron Cheletion, Gene Therapy, Bone Marrow Transplantation.

CHAPTER-1

BACKGROUND OF THE PROJECT

1.1 Introduction

The Ministry of Health and Family Welfare (MOH&FW) has approved the Programme Implementation Plan (PIP) of Health Population Nutrition Sector Development Programme (HPNSDP) with the vision of sustainable improvement in health, population and nutrition status of the people, particularly of vulnerable groups, including women, children, the elderly and the poor with an ultimate aim of their economic emancipation and physical, social, mental and spiritual well-being thus contribute to the poverty reduction strategies. The success of HPNSDP depends on the proper implementation of operational plans of different line programs under HPNSDP. The ultimate purpose of these operational plans is to reduce MMR, IMR, TFR, Malnutrition, Infant and under-5 mortalities, prevalence of communicable and non communicable diseases.

Bangladesh is a high prevalence country in consideration of Haemoglobin disorders. It is considered that among its 170 million people 4.1% (range 1-5%) are carriers of beta Thalassaemia, while 6.1% (range 4-10%) are carriers of the variant HbE. This means that an estimated 15000 new births of affected children are expected every year. A current estimate is that there may be up to 90000 patients with clinically significant Haemoglobin disorders living in the country at any time. Lack of adequate treatment will limit this number because of early mortality. In view of the fact, proven in many areas of the world, that proper treatment allows long survival with a fulfilling life in homozygous patients, the aim of any national policy should be to achieve this level of care, converting potentially handicapped patients to productive citizens.

The purpose of the study is to know about the Thalassaemia burden in Bangladesh and to develop a tool to diagnose Thalassaemia based on patient's economic characteristics.

1.2 Communicable Disease

Communicable diseases are diseases that are as a result of the causative organism spread from one person to another or from animals to people. The spread or transfer can happen through the air, through contact with contaminated surfaces, or through direct contact with blood, feces, or other bodily fluids. Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi; the diseases can be spread, directly or indirectly, from one person to another. Communicable diseases are preventable base on interventions placed on various levels of transmission of the disease.

Infectious diseases may include Vector borne diseases e.g. dengue fever, malaria, Protozoal diseases like diarrhea, hepatitis A & E, Typhoid fever; Water borne diseases: leptospirosis, Animal contact disease (zoonotic): rabies. New diseases are being experienced from time to time Such Diseases may include Ebola, Enterovirus D68, FluHantavirus, Hepatitis B, C, HIV/AIDS, Measles, MRSA, Pertussis, Rabies, Sexually Transmitted Disease, Shigellosis, Tuberculosis, West Nile Virus, Zika etc.

1.3 Non Communicable Disease

Non communicable diseases (NCDs) which include cardiovascular disease, diabetes, chronic respiratory diseases and certain cancers have become a global problem accounting for more than 68% of the total global deaths. All age groups are affected by NCDs although NCDs are more common in older age group. Key shared NCD risk factors include tobacco use, unhealthy diet,

physical inactivity, and harmful use of alcohol. Overweight/obesity, high blood pressure, raised blood sugar and raised blood lipids A are intermediate metabolic risk factors for NCDs. In Bangladesh, NCDs are an imminent public health issue. Currently three quarters of the population are exposed to two or more modifiable NCD risk factors and 5% of the adult population are diabetic, and 23% hypertensive.

NCD result in significant socio economic and health care costs and is detrimental to sustainable development. The chronic nature of the diseases requires protracted treatment and can lead to catastrophic expenditure particularly among the poor. NCDs affect both affluent and the poor. However, NCDs can affect the poor disproportionately leading into the vicious cycle of disease, poverty and non-productivity. Investments in NCD prevention are generally not commensurate with the high disease burden despite existence of proven cost effective public health interventions. Low cost solutions to NCD include modifying exposure to common NCD risk factors. Early detection of NCDs through a primary health care approach is a high impact intervention. The suggested broad approaches require partnership, leadership and commitment of many stakeholders beyond the health sector. Sectors such as local governments, urban planning, transport, education, agriculture, finance and NGOs therefore, have a great stake in NCD prevention. Although globally NCD refers to four common diseases, i.e., hypertension, diabetes, cancer and chronic obstructive pulmonary diseases, there are some other NCDs which also can be devastative. One of these is Haemoglobinopathies.

1.4 Haemoglobinopathy

Haemonoglobinopathy is a broad range of genetic disorder affecting the blood cells. It is characterized by decline, below the natural rate, in both the red blood cells and the level of Haemoglobin or other characteristic. Hemoglobin is a substance in the red blood cells that carries oxygen, therefore the decline of Haemoglobin triggers aenemia.

Different variants of Haemigobinopathies include:

1. Structural defects in the hemoglobin molecule. Alterations in the gene for one of the two hemoglobin subunit chains, alpha or beta*,* are called mutations. Often, mutations change a single amino acid building block in the subunit. Most commonly the change is innocuous, perturbing neither the structure nor function of the hemoglobin molecule. Occasionally, alteration of a single amino acid dramatically disturbs the behavior of the hemoglobin molecule and produces a disease state. Sickle hemoglobin exemplifies this phenomenon.
2. Diminished production of one of the two subunits of the hemoglobin molecule. Mutations that produce this condition are termed "thalassemias." Equal numbers of hemoglobin alpha and beta chains are necessary for normal function. Hemoglobin chain imbalance damages and destroys red cells thereby producing anemia. Although there is a dearth of the affected hemoglobin subunit, with most thalassemias the few subunits synthesized are structurally normal.
3. Abnormal associations of otherwise normal subunits. A single subunit of the alpha chain (from the α-globin locus) and a single subunit from the β-globin locus combine to produce a normal hemoglobin dimer. With severe α-thalassemia, the β-globin subunits begin to associate into groups of four (tetramers) due to the paucity of potential α-chain partners. These tetramers of β-globin subunits are functionally inactive and do not transport oxygen. No comparable tetramers of alpha globin subunits form with severe beta-thalassemia. Alpha subunits are rapidly degraded in the absence of a partner from the beta-globin gene cluster (gamma, delta, beta globin subunits).

Types of hemoglobins

There are hundreds of hemoglobin variants that involve involve genes both from the alpha and beta gene clusters. The list below touches on some of the more common and important hemoglobin variants.

Normal Hemoglobins

- Hemoglobin A. This is the designation for the normal hemoglobin that exists after birth. Hemoglobin A is a tetramer with two alpha chains and two beta chains $\alpha_2\beta_2$).
- Hemoglobin A2. This is a minor component of the hemoglobin found in red cells after birth and consists of two alpha chains and two delta chains $\alpha_2\ \delta_2$). Hemoglobin A2 generally comprises less than 3% of the total red cell hemoglobin.
- Hemoglobin F. Hemoglobin F is the predominant hemoglobin during fetal development. The molecule is a tetramer of two alpha chains and two gamma chains $\alpha_2\gamma_2$).

The genes for hemoglobin F and hemoglobin A are closely related, existing in the same gene cluster on chromosome 11. Hemoglobin F production falls dramatically after birth, although some people continue to produce small amounts of hemoglobin F for their entire lives.

Clinically Significant Variant Hemoglobins

- Hemoglobin S: This the predominant hemoglobin in people with sickle cell disease. The alpha chain is normal. The disease-producing mutation exists in the beta chain, giving the molecule the structure, $\alpha_2\beta_2{}^S$: People who have one sickle mutant gene and one normal beta gene have sickle cell trait which is benign.
- Hemoglobin C: Hemoglobin C results from a mutation in the beta globin gene and is the predominant hemoglobin found in people with hemoglobin C disease $\alpha_2\beta_2{}^C$: Hemoglobin C disease is relatively benign, producing a mild hemolytic anemia and splenomegaly. Hemoglobin C trait is benign.
- Hemoglobin E: This variant results from a mutation in the hemoglobin beta chain. People with hemoglobin E disease have a mild hemolytic anemia and mild splenomegaly. Hemoglobin E trait is benign. Hemoglobin E is extremely common in S.E. Asia and in some areas equals hemoglobin A in frequency.
- Hemoglobin Constant Sprin:. Hemoglobin Constant Spring is a variant in which a mutation in the alpha globin gene produces an alpha globin chain that is abnormally long. The quantity of hemoglobin in the cells is low for two reasons. First, the messenger RNA for hemoglobin Constant Spring is unstable. Some is degraded prior to protein synthesis. Second, the Constant Spring alpha chain protein is itself unstable. The result is a thalassemic phenotype. (The designation Constant Spring derives from the isolation of the hemoglobin variant in a family of ethnic Chinese background from the Constant Spring district of Jamaica.)
- Hemoglobin H: Hemoglobin H is a tetramer composed of four beta globin chains. Hemoglobin H occurs only with extreme limitation of alpha chain availability. Hemoglobin H forms in people with three-gene alpha thalassemia as well as in people with the combination of two-gene deletion alpha thalassemia and hemoglobin Constant Spring.
- Hemoglobin Barts: Hemoglobin Barts develops in fetuses with four-gene deletion alpha thalassemia. During normal embryonic development, the episilon gene of the alpha globin gene locus combines with genes from the beta globin locus to form functional hemoglobin molecules. The episolon gene turns off at about 12 weeks, and normally the alpha gene takes over. With four-gene deletion alpha thalassemia no alpha chain is produced. The gamma chains produced during fetal development combine to form gamma chain tetramers. These molecules transport oxygen poorly. Most individuals with four-gene deletion thalassemia and consequent hemoglobin Barts die in utero (hydrops fetalis). The abnormal hemoglobin seen during fetal development in individuals with four-gene deletion alpha thalassemia was characterized at St.

Bartholomew's Hospital in London. The hospital has the fond sobriquet, St. Barts, and the hemoglobin was named "haemoglobin Barts"

1.5 Thalassaemia

Thalassaemia, one form of Haemoglobinopathy, is present almost in every community. However, it is found to be more prevalent in Mediterranean countries, Middle East, Gulf regions, Northern parts of Africa, Central Asian Countries , and India, Bangladesh .Certain types of Thalassaemia are most common in particular parts of the world. The most common type of β-Thalassaemia is prevalent in the tropical and sub-tropical regions (**Weatherall and Clegg, 1996**).

Early genetic diagnosis, proper counseling, health education, community based prevention, screening before marriage, strong policy from Government are necessary for the improvement of this inherited disorder in Bangladesh. Majority are born in countries with limited resources where priority tends to be given to tackling high rates of infant and child mortality from infection diseases and malnutrition. Often affected children of severe Thalassaemia are not diagnosed properly and do not receive adequate treatment and die during childhood.

In world now almost 7% of the total world population is infected with Haemoglobin disorders, 300,000 – 500,000 children are born with Haemoglobin disorders, 70% of children are born with sickle cell anemia, and the rest are living with Thalassaemia. Every year, 50-80% of children die from the sickle cell anemia. Every year, 50,000 – 100,000 children die from major Thalassaemia over the globe.

Thalassaemia is the name of a group of genetically inherited blood disorder passed down through families in which the body makes an abnormal form of Haemoglobin, the protein in red blood cells that carries oxygen. It results in excessive destruction of red blood cells, which leads to anemia. **Patients have a lower-than-normal number of red blood cells in their bodies and too little Haemoglobin. In many cases the red blood cells are too small.**

These conditions cause varying degrees of anemia, which can range from insignificant to life threatening. It is not infectious and cannot be passed from one individual to the other by personal or any other contact, or through blood transfusion, food or air (**Wikipedia, 2008**).

Usual adult Haemoglobin is made up of three components: alpha-globin, beta-globin and haem.Thalassaemias are classified according to the globin that is affected, hence the names alpha and betaThalassaemia. Although both classes of Thalassaemia affect the same protein, the alpha and beta Thalassaemias are distinct diseases that affect the body in different ways.

1.5.1 Alpha Thalassaemia

The alpha Thalassaemia patient's Haemoglobin does not produce enough alpha protein. This type is commonly found in southern China, Southeast Asia, India, the Middle East and Africa. Signs and Symptoms of Alpha Thalassaemia:

- Fatigue
- Drowsiness
- Chest pain
- Pale skin
- Cold hands and feet
- Headaches
- Dizziness and feeling faint
- Shortness of breath

1.5.2 Beta Thalassaemia

For beta Thalassaemia two globin genes need to make beta globin chains. We get one from each parent. If one or two of these genes are faulty, it produces beta Thalassaemia. Beta Thalassaemia is much more common among people of Mediterranean ancestry, hence its other name, Mediterranean anemia. It is also more prevalent in North Africa and West Asia. Sixteen percent of the people in the Maldives, some islands in the Indian Ocean, are carriers.

The majority of infants with beta Thalassaemia will not have symptoms until they reach six months, because they start off with a different type of Haemoglobin called fetal Haemoglobin. After the age of six months "normal" Haemoglobin starts replacing the fetal one. People with Thalassaemia mainly have anemia-like symptoms. Other symptoms are:

- Pale skin
- Jaundice
- Fatigue
- Cold hands and feet
- Shortness of breath
- Poor feeding
- Delayed growth
- Skeletal deformities - in some cases as the body tries to produce more bone marrow
- Too much iron - the body will try to absorb more iron to compensate. Iron may also accumulate from blood transfusions. Excessive iron can harm the spleen, heart and liver
- Greater susceptibility to infections
- Delayed puberty

Epidemiology

Thalassemia is considered the most common genetic disorder worldwide(Illeymanet al ., 2000).Thalassemia is found in some 60 countries with the highest prevalence in the Mediterranean region, parts of North and West Africa, the Middle East, the Indian subcontinent, southern Far East and southeastern Asia, especially, Thailand and southern China together composing the so-called thalassemia belt(**Hoffman et al., 2005**).In Asia, the highest incidence of thalassemia found in Maldives with a carrier rate of 18% of the population(**Furuumiet al., 2006**).

The estimated prevalence is 16% in people from Cyprus(**Yaish, 2007**),in Thailand 1%are affected and more than 20 million were thalassemia carriers(**Chonnanitet al. ,2005**)and 3-8% in populations from Bangladesh , China , India , Malaysia and Pakistan. (**Hoffmanet al., 2005**).

In Europe, the highest concentrations of the disease are found in Greece, including the Greek islands; in parts of Italy, lower Po valley(**Peres et al., 1996**); in southern Italy; and in the Italian islands Sicily, Sardinia (**Guiso et al., 1996**) , and Malta Corsica (**French island**) and Crete (**Greek islands**)(**Hoffmanet al., 2005**).A very low prevalence has been reported from people in northern Europe (0.1%) and Africa (0.9%)(**Ballas et al., 1997**).

The highest frequency of the alpha thalassemia genes is found in Southeast Asia(Ko and Xu , 1998),Africa(**Ballas et al., 1997**)and in Mediterranean region including Portugal with incidence of α - thalassemia carriers is (10%)(**Peres et al., 1996**), (18%) in Sardinians(**Guiso et al.,1996**), and 7% in Greece(**Hoffmanet al., 2005**).

The population of northern Thailand, with a prevalence of about 5% to 10%,Harbors one of the highest incidences of α-thalassemia in the world (**Chonnanitet al. ,2005**).

About 150 million people worldwide carry ß-thalassemia genes. Beta thalassemias are distributed widely in Many Mediterranean islands, including Cyprus (**Yaish, 2007**), Sardinia (11-34%) (**Hoffmanet al., 2005**) and Sicily (10%), have a significantly high incidence of severe beta thalassemia, constituting a major public health problem(**Hoffmanet al., 2005**). For instance, in Cyprus, 1 in 7 individuals carries the gene, which translates into 1 in 49 marriages between carriers and 1 in 158 newborns expected to have β thalassemia major(**Yaish, 2007**).

The genes are particularly prevalent in Italy and Spain. Other regions with the high gene frequency are Greece (5-15%)(**Hoffmanet al., 2005**), Iran (4-10%) (Zlotogora, 1995), and Thailand (1-9%) (**Chonnanitet al, 2005**). Many of β-thalassemia gene mutations detected in Turkish (**Hoffmanet al**., **2005**), Kurdish(**Cohen and Filon, 1991**), Bulgarian (**Hoffmanet al., 2005**), Asian Indian origin (**Vaz et al., 2000**) and Pakistan (**Vaz et al., 2000**).The β thalassemias are rare in Africa, except for some isolated pockets in West Africa, notably Liberia, and in parts of North Africa (**Ballas et al., 1997**).

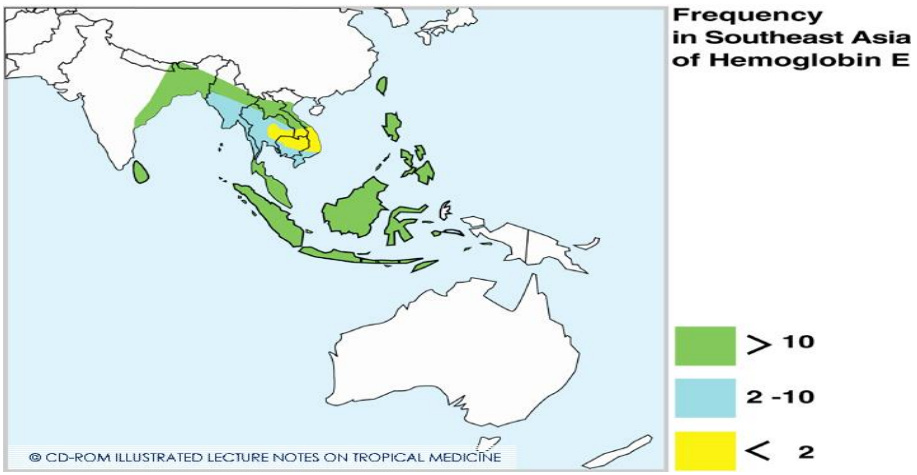
1.6 HB E- Trait and Disease

Hemoglobin E disease is a recessively inherited genetic blood disorder characterized by abnormally small red blood cells which generally causes no health problems. Some people may suffer mild hemolytic anemia or a slightly enlarged spleen.

Definition:
Hemoglobin E =HbE ($\alpha 2\beta 2^{26\ Glu\_Lys}$) is a variant hemoglobin with a mutation in the β globin gene causing substitution of glutamic acid for lysine at position 26 of the β globin chain. HbE may be present in the heterozygous state (genotype AE or hemoglobin E trait), the homozygous state (EE or hemoglobin E disease) and a variety of compound heterozygous states such as hemoglobin E/β thalassemia (E/βthal), sickle cell/hemoglobin E disease (SE genotype).

The β chain of HbE (β^E) is synthesized at a reduced rate compared with that of normal adult hemoglobin (Hb A). This is because the mutation creates an alternate splicing site within an exon. This results in reduced rate of synthesis of β^E chain and therefore of HbE, and consequently heterozygotes, compound heterozygotes and homozygotes show some β thalassemic features. HbE may therefore be regarded as a β^+thalassemic hemoglobinopathy.

Epidemiology:



HbE is the second commonest abnormal hemoglobin after sickle cell hemoglobin (Hb S). HbE is common in South-East Asia. The highest prevalence of carriers is in some parts of Thailand, in Cambodia and in Laos. Thailand and Myanmar (previously Burma) have an overall prevalence of

around 14-15 %. Gene frequency in Thailand varies from 8 to 50-70 %, being highest in North-Eastern Thailand. Hemoglobin E is also found in Sri Lanka, North Eastern India, Bangladesh, Pakistan, Nepal, Vietnam, Malaysia, the Philippines, Indonesia and Turkey.

Included diseases:
Hemoglobin E trait, hemoglobin E disease, hemoglobin E/β thalassemia, sickle cell/hemoglobin E disease

Heamoglobin E trait
Clinical features
HbE trait is an asymptomatic condition with no clinical relevance, except for the risk of compound heterozygous states with β thalassemia in the offspring.

Laboratory features
Most of individuals with HbE trait have reduced mean corpuscular volume (MCV) and mean cell hemoglobin (MCH), with or without mild anemia. The red blood cell indices resemble those of thalassemia trait. Individuals with HbE trait may carry α thalassemia conditions of varying severity according to the number of non-functional α genes 1, 2, 3. However even those with a full complement of α genes may be microcytic and mildly anemic.

The blood film may be normal or may show hypochromia, microcytosis, target cells, irregularly contracted cells, basophilic stippling or any combination of these features.

Electrophoretic Profile
Hemoglobin electrophoresis at alkaline pH on cellulose acetate shows that the variant HbE has the same mobility than that of the variant HbC (α2 β2 $^{6glu\_lys}$) and the adult hemoglobin HbA2 (α2 δ2). On citrate agar or agarose gel at acid pH, the mobility of HbE is the same than that of HbA and HbA2. HbE has a characteristic mobility on isoelectric focusing, being well separated from HbA and focusing like HbA2, and thus unlike HbC. On HPLC, HbE is easily separated from HbA and HbC, but may co elute with HbA2.

In HbE heterozygotes, the variant usually comprises 33 % or less of total HbA. Individuals with less than 30% of HbE almost always have co existing α thalassemia trait.
HbE is slightly unstable in heat and isopropanol stability tests.

Hemoglobin E Disease
Hemoglobin E disease is defined by the coexistence of two βE alleles (homozygous state EE). At birth, differential diagnosis is E/βthalassemia, which is always symptomatic after disappearance of HbF. Study of both parents (heterozygotes AE in case of homozygous EE in the child) is mandatory.

Clinical Features
Individuals with the genotype EE are usually completely asymptomatic. There is usually no anemia and rarely any evidence of hemolysis. The spleen is not usually enlarged. Otherwise a coexisting HbH disease (α_3 thalassemia) must be considered.

Laboratory Features
Blood count: The blood count often resembles that of β thalassemia trait, with a normal hemoglobin concentration or very mild anemia and increased red blood cell (RBC), reduced MCV and MCH. MCHC is usually normal.

Blood film: The blood film usually shows hypochromia and microcytosis with variable numbers of target cells, basophilic stippling and irregularly contracted cells.

Electrophoretic profile
Hemoglobin electrophoresis shows the major hemoglobin to be HbE, with HbE plus HbA_2 constituting 95-99 % of total hemoglobin.

Hemoglobin E - β Thalassaemia
Hemoglobin E trait may be co inherited with either β^0 or β^+ thalassemia. The compound Asia, stretching from Indonesia to Sri Lanka, North-East India and Bangladesh, making this the most common β thalassemia phenotype in the world and one of the most prevalent genetic diseases in the world.

Clinical Features
The severity of compound heterozygotes for HbE and β thalassemia is very variable, the clinical picture ranging from that of β Thalassemia minor through β thalassemia intermedia to β thalassemia major. Most patients have a disease that is at least moderately severe.

The most severely affected individuals are transfusion dependent and have liver enlargement and splenomegaly, intermittent jaundice, growth retardation and over expansion of the bone marrow cavity leading to facial deformity and defective tooth implantation. Less severely affected individuals may have splenomegaly and facial deformity but do not require regular transfusions to maintain life. Hypersplenism may occur. Splenectomy is usually efficient in reducing transfusion requirements. During pregnancy, patients may temporarily become transfusion dependant. Extramedullary hemopoiesis can occur and has sometimes led to compression of the spinal cord or brain by tumour like masses of hemopoietic tissue.

Blood Count
The hemoglobin concentration is lower than in HbE sease. Hydroxyurea therapy alone or with recombinant erythropoietin may induce a slight rise in hemoglobin concentration, but sufficient to avoid transfusion need.

Other Investigations
Hemoglobin electrophoresis and HPLC show the presence of HbE, HbA2 and HbF in the case of HbE/β^0 thalassemia, and HbE, HbA, HbA2 and HbF in the case of HbE/β^+ thalassaemia. When HbA is present, it usually represents around 10 % of total hemoglobin.

1.7 Importance of Studies on Haemoglobinopathy

Bangladesh is a high prevalence country where Haemoglobin disorders are concerned. Among its 170 million people 4.1% (range 1-5%) are carriers of Beta Thalassaemia, while 6.1% (range 4-10%) are carriers of the variant HbE. Though HbE causes of aenemia and HbE trait has no apparent effect on an individual. When Hb E trait and beta trait combine in an offspring from two traits of mother and father, HbE/Beta Thalassaemia occurs. This can be as severe as Beta Thalassaemia.

This means that an estimated 14500 new births of Beta Thalassaemia and HbE-Beta Thalassaemia children are expected every year. In patients taking blood transfusion in Bangladesh Thalassaemia Hospital(BTH) about 30% are Beta Thal and 70% are HbE-Beta Thal patients. A current estimate is that there may be up to 90000 patients with clinically significant Haemoglobin disorders living in the country at any time. Lack of adequate treatment will limit this number because of early mortality. In view of the fact, proven in many areas of the world, that proper treatment allows long survival with a

fulfilling life in homozygous patients, the aim of any national policy should be to achieve this level of care, converting potentially handicapped patients to productive citizens.

The government in its Vision 2021 the government seeks to create conditions whereby the people of Bangladesh have the opportunity to reach and maintain the highest level of attainable health. The 7th Five year goals includes meeting challenges of emerging and re-emerging and non-communicable diseases, health hazards due to climate change and emergency response to catastrophe.

Haemoglobinopathy being a non communicable disease having immense impact on the society needs to be addressed properly. It requires blood transfusion and medicine throughout the life otherwise whole resources will be simply a waste as early death may occur. Only full treatment will give value for money.

Therefore a national program should include a policy of prevention with a view to limiting new births with the disease. This study will therefore throw light on the prevailing situation and future needs.

CHAPTER-2

UNDERSTANDING OF THE PROJECT AND THE ASSIGNMENT

2.1 Objectives of the Assignment

Countries such as Nepal, Bangladesh and Pakistan are seeing a large increase of thalassaemia patients due to lack of genetic counseling and screening. In Bangladesh data is not enough to suggest its prevalence rate. Concern is increasing that thalassaemia may become a very serious problem in the next 50 years, one that will burden the world's blood bank supplies and the health system in general. Taking into consideration the government is contemplating to take measures to prevent the progression of the disease. The objectives of assignment are to obtain information addressing the following specific parameters:

- Estimate Thalassaemia prevalence in Bangladesh
- Describe the characteristics of the patients.
- Develop an economic screening tool to diagnose thalassaemia in Bangladesh.

2.2 Scope of Work

In view of attaining the objective the following scope of works has been set in the Terms of Reference (TOR):

Review of literature
Review existing literature available globally and nationally on Thalassaemia.
Develop a tool which may diagnose Thalassaemia based on economic background of a probable patient
Complete the assessment and submit a report

2.3 Output of Assignment

In view of Terms of Reference (TOR) following are the output of the assignment:
Inception Report
Progress report
Mid-term Progress report
Soft copy of primary data
Draft Final report
Final Report

2.4 Heamoglobinpathy: Global Perspective

Hemoglobinopathy is a kind of genetic defect that results in abnormal structure of one of the globin chains of the haemoglobin molecule. Hemoglobinopathies are inherited single-gene disorders; in most cases, they are inherited as autosomal co-dominant traits. Common hemoglobinopathies include sickle-cell disease. It is estimated that 7% of world's population (420 million) are carriers, with 60% of total and 70% pathological being in Africa. Hemoglobinopathies are most common in ethnic populations from Africa, the Mediterranean basin and Southeast Asia.

Hemoglobinopathies imply structural abnormalities in the globin proteins themselves.Thalassemias, in contrast, usually result in underproduction of normal globin proteins, often through mutations in regulatory genes. The two conditions may overlap, however, since some conditions which cause

abnormalities in globin proteins (hemoglobinopathy) also affect their production (thalassemia). Thus, some hemoglobinopathies are also thalassemias, but most are not.

Either hemoglobinopathy or thalassemia, or both, may cause anemia. Some well-known hemoglobin variants such as sickle-cell anemia and congenital dyserythropoietic anemia are responsible for diseases, and are considered hemoglobinopathies. However, many hemoglobin variants do not cause pathology or anemia, and thus are often not classed as hemoglobinopathies, because they are not considered pathologies. Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Other variants cause no detectable pathology, and are thus considered non-pathological variants. Different variants identified are listed below:

Hemoglobin Variants

- Hb S
- Hb C
- Hb E
- Hb Bart's
- Hb D-Punjab
- Hb O-Arab
- Hb G-Philadelphia
- Hb Constant Spring
- Hb Hasharon
- Hb Korle-Bu
- Hb Lepore
- Hb M
- Hb Kansas

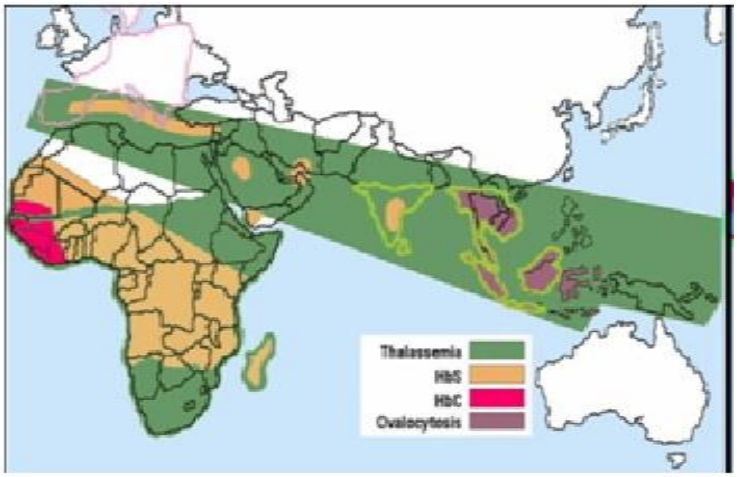
An estimated 60-80 million people in the world carry the β-Thalassaemia, a type of Haemoglobinopathy, trait. This is a rough estimate; the actual number of those with **Thalassaemia major** is unknown due to the prevalence of Thalassaemia in less-developed countries.

The beta form of Thalassaemia is particularly prevalent among **Mediterranean** peoples, and this geographical association is responsible for its naming. In Europe, the highest concentrations of the disease are found in **Greece**, coastal regions in **Turkey**, in parts of **Italy**, particularly **Southern Italy** and the lower Po Valley. The major Mediterranean islands such as **Sicily**, **Sardinia**, **Malta**, **Corsica**, **Cyprus,** and **Crete** are heavily affected in particular. Other Mediterranean people, as well as those in the vicinity of the Mediterranean, also have high rates of Thalassaemia, including people from **West Asia** and North Africa. Far from the Mediterranean, **South Asians** are also affected with the world's highest concentration of carriers (16% of the population) being in the **Maldives**.

Nowadays, it is found in populations living in Africa, the Americas, and in Tharu people in the **Terai** region of **Nepal** and **India**. It is believed to account for much lower malaria sicknesses and deaths, accounting for the historic ability of Tharus to survive in areas with heavy malaria infestation, where others could not. Thalassaemias are particularly associated with **Arabs** (especially **Palestinians** and people of Palestinian descent). The estimated prevalence is 16% in people from **Cyprus,** 1% in **Thailand**, and 3–8% in populations from **Bangladesh**, **China**, **India**, **Malaysia** and **Pakistan**.

The global scenario in short depicts that:

- 1.5% (80-90 million people) of the world's population is carriers of β Thalassaemia and 5% are carriers of α Thalassaemia.
- β Thalassaemia is prevalent in areas around the Mediterranean, in the Middle East, in Central, South, and Southeast Asia, and in Southern China. Table 2.1 below depicts the situation country wise.
- β Thalassaemia and sickle cell is prevalent in America as given in Table 2.2
- β Thalassaemia and sickle cell is prevalent in Europe as given in Table 2.3
- β Thalassaemia and Hb E is prevalent in Southeast Asia, Asia Pacific and China as depicted in Tbale 2.4.
- Increasing migration of populations at risk to non-endemic countries has resulted in increasing prevalence of Thalassaemia gene mutations in all parts of the world.



Occurrence of Thalassaemia over the Globe

East Mediterranean Region

Table 2.1 Prevalence Mediterranean Region

Country	Population millions	% HbS carriers	Annual SCD Births expected	% ß-thal. Carriers	Expected homozygote births/year	Known ß-thal. homozygotes	Known SCD patients
Afghanistan	28.4	0	0	3	269	na	na
Algeria	35.7	1.25	100	2	61	3000	4711
Bahrain	1.2	11	61	3.5	4	300	600
Egypt	82.08	0.2	109	5.3	1423	9912	1166
Iran	77.9	1	371	6	1027	18616	na
Iraq	30.4	0.7	157	4.8	502	10808	3620
Jordan	6.5	1.5	40	3.5	38	1300	150
Kuwait	2.6	6	100	3	12	129	500
Lebanon	4.14	2	28	3.5	19	678	387
Libya	6.6	2	40	1.5	9	na	na
Morocco	34.8	1.76	164	1.67	51	375	na
Oman	3.02	5.7	105	3.1	16	591	4704
Pakistan	187.3	0.27	356	6	4600	50000	na
Palestine Gaza	1.7	1	14	4.2	26	162	na
Palestine West Bank	3.8	1.5	31	3.8	33	526	131
Qatar	0.84	5	18	3	3	146	280
Saudi Arabia	26.1	4.2	473	2.37	184	600	26000
Sudan	45.04	2.25	917	3.9	617	na	na
Syria	22.5	0.5	71	5	338	7700	100
Tunisia	10.3	1.9	49	2.21	20	742	1526
UAE	5.1	1.1	36	8	123	1000	na
Yemen	24.1	2.2	489	4.4	391	800	na

Abbreviations: SCD, sickle cell disease; thal, Thalassaemia; homoz, homozygote/s

Table 2.2 Prevalence in America

Country	Population millions	% HbS carriers	Expected SCD births/year	% β-thal. Carriers	Expected β-thal births/year	Known β-thal patients	Known SCD patients
Argentina	41.8	0.7	44	1.35	33	na	na
Bahamas	0.316	10	15	1	<1	na	na
Barbados	0.29	7	5	0.5	<1	na	na
Brazil	203.4	4	2417	1.3	155	na	25-50000
Canada	34.3	0.3	3	0.5	4	300	na
Colombia	45.2	2.4	187	0.8	12	na	na
Cuba	11.05	3.08	39	0.75	2	na	4000
Dominican Republic	10.1	4.8	160	1	5	na	na
Haiti	9.8	15	1492	1	6	na	6000
Jamaica	2.89	10	177	1.5	3	na	na
Trinidad & Tobago	1.22	9.9	74	3.5	7	63	na
USA	312.8	0.75	115	0.4	24	716	70-100000
Venezuela	28.04	1.96	63	0.2	1	na	na

Abbreviations: SCD, sickle cell disease; thal, Thalassaemia; homoz, homozygote/s

Table 2.3 Prevalence in Europe

Country	Population millions	Immigrants (%) from at risk populations	% β-thal. Carriers immigrants + indig	% HbS carriers immigrants + indig	Expected β-thal Births/year if no prevention	Known β-thal. homozygotes	Known SCD patients	Known β-thal patients
Albania	3.2		5	1.4	18	12	530	500
Armenia	2.97		2	0	4	0	0	199
Austria	8.2	4.70%	0.24	0.06	<1	<1	na	na
Azerbaijan	9.5		8	0	263	0	0	642-2000
Belgium	10.4	3.80%	0.28	0.42	<1	1	358	62
Bosnia	4.61		1.2	0	1-2	0	0	na
Bulgaria	7.36	0.40%	2.5	0	11	0	0	270
Croatia	4.5		0.8	0	1	0	0	na
Cyprus	0.84	9.60%	14.4	0.27	52	2	40	681
Czech Rep	10.3	0.8	0.12	0.002	<1	0	0	na
Denmark	5.5	4.70%	0.26	0.11	<1	<1	na	26
France	64.05	4.80%	0.7	0.62	12	26	10000	378
Georgia	4.61		3	0	11	0	na	na
Germany	82.3	5.50%	0.28	0.06	1	1	1500	1600
Greece	10.7	5.80%	8.1	0.6	167	26	1080	3241
Ireland	4.2	1.30%	0.13	na	<1	<1	na	na
Israel	7.59		0.5	1	1	7	70	373
Italy	58.1	4.30%	4.3	2.1	220	267	829	6000
Kazakhstan	15.5		1	0	6	0	0	na
Kyrgyzstan	5.6		1.5	0	7	0	0	na
Luxenburg	0.49	22.80%	0.5		<1	<1	na	na
Malta	0.4		3		1			21
Netherlands	16.7	7.30%	0.4	0.18	1	1	616	140
Norway	4.66	4.80%	0.3		<1	<1	na	48
Portugal	10.7	3.44%	1.44	1.13	6	12	500	40
Romania	21.9	0.30%	1	0.002	5	0	0	200-300
Russian Fed.	147.2	2.30%	0.18		1	<1	na	na
Serbia	7.38		1.2		2	<1	na	na
Slovenia	20.5	5.90%	0.18		<1	<1	na	na
Spain	40.5	5.40%	1.64	0.3	27	11	350	27
Sweden	9.05	5.40%	0.32	0.09	<1	<1	100	50
Switzerland	7.6	13.80%	0.4		<1	<1	na	na
FYR Macedonia	2.06		2.6	0	4	<1	na	na
Turkey	79.7		2.2	0.44	173	76	1050	3264
Turkmenistan	5.05		1.2	0	4	0	na	na
United Kingdom	62.6	8%	0.44	2.5	4	142	12000	900
Uzbekistan	28.6		3	0	112	0	na	188

Abbreviations: SCD, sickle cell disease; thal, Thalassaemia; homoz, homozygote/s; indigenous

Table 2.4 Prevalence in Asia

Country	Population millions	% HbE carriers	% β-thal carriers	Expected β-thal syndromes Births/year	Known patients
East Asia					
Bangladesh	161.1	6.1	4.1	5477	6880
Bhutan	0.7	4	na	na	na
India	1270.0	1	3.9	16200	100000
Maldives	0.4	0.9	18	54	574
Myanmar	54.6	22	2.2	2398	4079
Nepal	29.4	4.4	4	836	400
Sri Lanka	21.5	0.5	2.2	64	3410
Brunei Darussalam	0.4		2	1	179
Indonesia	245.6	6(1-25)	5(3-10)	9619	5000
Thailand	67.1	30	5	6983	35000
Timor Leste	1.2	6	5	65	na
Asia Pacific					
Cambodia	14.7	30	3	1762	na
Lao Peop.Dem.Rep.	6.6	18	6	1106	na
Malaysia	28.7	3.4	4.5	727	1500
Philippines	101.8	0.4	1.2	153	600
Singapore	4.7	0.64	3	13	154
Austarlia	21.6	0.4	0.4	3	332
Viet Nam	90.5	1	2.6	424	1000
Taiwan	22.9	0.027	2	21	400
Chinese territories					
Hong Kong	7.05	0.3	3.5	19	363
Guanxi	49.2	0.42	6.78	902	na
Guangdong	91.9	0.06	2.54	182	na
Yunnan	44.5	1.6	3.7	418	na
Guizhu	39.3	na	1.1	17	na
Sichuan	87.2	na	2.18	94	na
Hainan	82.8	na	2.09	132	na
Macau	0.55	na	3	2	na
Fujian	36	na	1.32	18	na

Abbreviations: thal, thalassaemia

2.5 Heamoglobinopathies: Bangladesh Perspective

The prevalence rate of haemoglobinopathies in Bangladesh is not well documented. A study on Bangladeshis living in UK is cited as a document by WHO. WHO data shows carrier status of Beta Thalassaemia trait to be 3% & Hb E trait to be 4%. However, much of the data available from WHO was based on studies that were carried many years ago and no regional data are available and there is no data base also.

In 2003 the Bangladesh Thalassaemia Samity could get some fund from Canadian International Agency (CIDA) to upgrade the facilities of the hospital. Part of the fund was allowed by CIDA to conduct a survey. Thus, with this fund a total number of 1248 samples of blood were collected from high school and college students during the last 6 months of 2003. More that 50% of the samplelwere from girls.The result from the study showed that average Hb E trait 5.17% and Beta Thalassaemia trait 4.20% in Bangladesh. Moreover Regional data showed that in Dhaka division Hb E 4.27%, Beta Carrier 3.61%, Chittagong division Hb E trait 6.42%, Beta Carrier 5.52%, Rajshahi division Hb E trait 8.69%, Beta Carrier 12% Khulna division Hb E trait 8.53%, Beta Carrier 4.87% ,Barisal division Hb E trait 2.9%, Beta Carrier 0, Sylhet division Hb E trait 0 & Beta Carrier 6.67% .Another study was done by Dr. Waqar Ahmed khan& others to determine the prevalence of Beta Thalassaemia trait and Hb E trait in school children of Bangladesh and calculate the health burden

of Thakassaemia.Haemoglobin Electrophoresis was done on blood collected from 735 school children from all six divisions of Bangladesh.

Over all prevalence of Beta Thalassaemia trait was 4.1% and Hb E trait was 6.1% in Bengali school children. The prevalence of Beta Thalassaemia trait in Bengali school children in Barisal division was 8.1% which was the highest followed by Rajshahi division 5.5%, Sylhet division 5.2%, Dhaka division 3.2%, Chittagong 2.9% & khulna division 2.4%. In tribal school children of Chittagong the prevalence rate of beta trait was 4.2%. Prevalence of Hb Etrait was 16.5% in Rajshahi division which was highest in Bengali school children followed by Barisal with prevalence of 8.1%, Dhaka 8%, Sylhet 4.2%, Chittagong 2.9% and khulna 2.4%. Prevalence of Hb E was 41.7% in tribal school children and 4.2 % were beta-thalassaemia carrier.

Recent epidemiological data published by TIF shows that Bangladesh is one of the most highly affected country with heamoglobin disorder in Asia with the carrier and frequency rates

a) Thalassaemia Trait – 4.1 %
b) Heamoglobin E trait – 6.1 %
c) Anticipated new affected births annually: 6435 based on carrier rate, population size and other demographic indicies.
d) Living patient with thalassaemia – 50,000 to 60,000.

2.6 Diagnosis of Thalassaemia and other Haemoglobinopathies Trait & Disease

Various screeing tools have been used to diagnose Thalassaemia and other Heamoglobinopathies. Sensitivity, Specificity and the Accuracy, positive predictive value and Negative value varies from method to method. Various methods followed are:

- Complete Blood Count (CBC)
- Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT)
- Di-chlorophenol Idophenol Precipitation (DCIP)
- Isoelectric Focusing (IEF)
- High performance chromatography (HPLC)
- Cation-exchange High performance chromatography (CE-HPLC)
- Capillary Electrophoresis (CE)

2.7 Treatment of Haemoglobinopathies

People with severe Thalassaemia require medical treatment. A blood transfusion regimen was the first measure effective in prolonging life. People with Thalassaemia can get an overload of iron in their bodies, from frequent blood transfusions. Too much iron can result in damage to the heart, liver, and endocrine system, which includes glands that produce hormones that regulate processes throughout the body. The damage is characterized by excessive deposits of iron. Without adequate iron chelation therapy, almost all patients with beta-Thalassaemia accumulate potentially fatal iron levels. Congestive heart failure and abnormal heart rhythms are also seen among Thalassaemia patients. People with Thalassaemia have an increased risk of infection. This is especially true if the spleen has been removed. Thalassaemia can make the bone marrow expand, which causes bones to widen. This can result in abnormal bone structure, especially in the face and skull. Bone marrow expansion also makes bones thin and brittle, increasing the risk of broken bones. Thalassaemia is frequently accompanied by the destruction of a large number of red blood cells, due to their abnormal structures. The task of removing these cells causes the spleen to enlarge. Splenomegaly can make anemia worse, and it can reduce the life of transfused red blood cells. Severe enlargement of the spleen may necessitate its removal. Anemia, caused by Thalassaemia may reduce a child's growth and puberty also may be delayed.

Bone marrow transplantation may offer the possibility of a cure in young people who have an **HLA**-matched donor. Success rates have been in the 80–90% range. Mortality from the procedure is about 3%. There are no randomized controlled trials which have tested the safety and efficacy of non-identical donor bone marrow transplantation in persons with β- Thalassaemia who are dependent on blood transfusion. If the person does not have an HLA-matched compatible donor, another method called bone marrow transplantation (BMT) from haploidentical mother to child may be used. In a study of 31 people, the Thalassaemia-free survival rate 70%, rejection 23%, and mortality 7%. The best results are with very young people.

2.8 Study Requirement:

The scope of study requires intense literary reviw and analysis. Support for field activity is ensured in the ToR. In the proposal, in consideration of obtaining some primary data has been proposed as a part of a pilot study.

CHAPTER-3

APPROACH AND METHODOLOGY

3.1 Approach of Task Identification

Our approach to the study was attainment of objectives. This involved analysis global literature down loaded from the internet, collection of local research results, though very small. As the time and scope of primary data collection is limited, data from secondary sources on trait cariers and patients were collected. To determine the prevalence of patients equation developed based on traits Vs paients have been used. Data collected were analysed to arrive at findings using simple excel data base. From the primary sample a certain portion has been tested for one more method in addition to electrophoresis to identify the efficacy of different methods. We have consulted the Line Director, Non Communicable Diseases Control Programme, from time to time and appraized him of the development.

3.2 Tasks Performed

The tasks performed are listed below:

- Collection and Review of local Literature
- Review of Global literature
- Comparative analysis of past after measures taken to prevent occurrence
- Relevance of Research and services at local and global level
- Design of Data collection Instruments
- Sample Design
- Field Survey Planning for Primary Data
- Screening tools now used in the country
- Scope of Use of Economic tool in the trait/ disease identification
- Collection of primary data and datxa from Secondary Sources
- Qualitative Data Collection
- Develop data management plan
- Data compilation and entry into the computer
- Data analysis and production of output tables
- Submission of Draft Report for comments
- Finalization and submission of reports incorporating comments & suggestions
- Submission of the Final Report

3.3 Methodology

3.3.1 Collection, Review and Analyses of Local and Global Literature

The consultants so far endeavored to collect relevant documents through Internet searching. The documents have been downloaded for Asian and global perspectives. This was followed by review and analysis. The followup activities were.

3.3.3.1 Comparative analysis of past after measures taken to prevent occurrence. This included the analyses of actions taken globally.

3.3.1.2 Relevance of Research and services at local and global level: This included abreif review of lacal situation with global perspective.

3.3.2 Development of Data Collection Instruments

Collection of data involved both primary and secondary data. The secondary data included data on Trait identification and determining patient characteristics.
Primary data collection format is given at Appendix 1
Secondary Trait detection survey format at Appendix 5
Data collection Format for Patient Characteristics at Appendix 4

3.3.3 Sample Design

The primary survey was conducted to gather opinion on awareness and prevalence of traits on sample basis in Dhaka city. The sample size was determined considering 95% confidence level and +/- 10% error limit. We drew sample from population falling within 15- 20, 21- 25, 25 – 35 and 35 -40 age limits. Each group was considered as a domain of study. Thus, four domains were considered for primary data collection for the study. But this is very small for determining the national characteristic having regional and ethnic variation. The time and scopes of works did not permit for larger sample and this may be considered to be pilot survey.

The approximate relationship between the sample size, n, and the proportion P is given by the following formula when finite population correction (fpc) is ignored and assuming Simple Random Sampling (SRS) design, is given by

$n_o = t^2 pq/d^2$

and with fpc

$n = n_o/[1+ (n_0 /N)]$

where n_o = Approximate sample size ignoring fpc
n = Sample size with fpc
p = Sample estimate of population proportion P
q = 1-p
t = Normal deviate corresponding to the confidence level is 95%
d = Level of accuracy or error margin one is willing to tolerate. It is generally 5 to 10 percent.
N = Size of population

It is further known that the variance pq is maximum, 0.25 when the proportion p=0.5 and thus for t=1.92 for 5% level of significance and d=10% margin of error.

$n_o = (1.92x0.5)/(0.1)^2 = 96$

The samples of these four domains will be collected from students/teachers of different types of education institutions given below. Thus the sample area will have (4X96)=384 sample persons.

3.3.4 Development of Field Survey Plan for Primary Data

For collection of primary data Dhaka City was divided in two parts, Dhaka North and Dhaka South. The survey plan was developed and survey conducted. The survey plan followed is given in Table 3.2 below:

Table 3.1: Quantitative Survey Plan Showing Distribution of Institutes and Volunteers

Sl No.	Dhaka North		Dhaka South		Remarks
	No of Institutes	No of Volunteers	No of Institutes	No of Volunteers	
1	1 school	25	1 school	25	The survey was conducted during the month of April and May on Randomly selected persons
2	2 colleges	50 (25 from each)	2 colleges	50 (25 from each)	
3	2 Universities	50 (25 from each)	2 Universities	50 (25 from each)	

Sl No.	Dhaka North		Dhaka South		Remarks
	No of Institutes	No of Volunteers	No of Institutes	No of Volunteers	
4	2 universities	50 (25 from each)	50 (25 from each)	50 (25 from each)	
5	1 University	25	1 University	25	

3.3.5 Collection of Primary Data

The teams were sent to the desired destination according to the prepared programme. Blood samples and household information on awareness etc were collected from the volunteers during the survey in the selected institutions. Blood samples were tested as per design following standard practice meeting the health service requirement.

In order to have an idea of local service available in respect of quality and quantity the following programmes at Table 3.3 were followed to identify the efficacy and reliability of test methods.

Table 3.2: Sample Distribution for Different Types of Testing Methods

Sl No.	Date	No of Samples	Remarks
1	NESTROFT/ Hb Electrophorasis	105	Randomly selected
2	Hb Electrophorasis	90	Randomly selected
3	Hb Electrophorasis	99	Randomly selected
4	Hb Electrophorasis +CBC	106	Randomly selected
5	Total	395	Randomly selected

3.3.6 Collection of Secondary Data on Prevalence of Trait

Survey on Thalassaemia trait prevalence was carried out by Bangladesh Thalassaemia Samity (BTS), The data were collected using the format given at Appendix 5.

3.3.7 Collection of Data on Patient Characteristics.

Checklist developed was used in the collection of data on patients coming at the following Institutions. The data collected from these secondary sources would give characteristics based on socio-economic, physical and psychological conditions of the patients.

- Bangladesh Thalassaemia Samity (BTS) & Hospital
- Bangladesh Shishu Hospital

3.3.8 Collection of Qualitative Data

Focus Group Discussions (FGDs)

In all 4 FGDs were conducted in under this study. The checklist developed for conducting FGD to collect information from stakeholders who may include professors/Teachers of University/ college/Schools will be used in the discussion sessions. Each session will be continued for 1-2 hours with 8-12 participants. Checklist given at Appendix 3 was used for holding discussion.

Key Informant Interviews (KII*)*

We conducted KII with Medical Professionals expert in Thalassaemia and patient management. We will conduct a total of 10 key informant interviews (KIIs) under this assignment. Checklist given at Appendix 2 was used for holding interview.

3.3.9 Data Management

Data collected were simple and straight forward. Data entry formats for entry of data were made in Ms Excel.

Data collected from Primary, secondary sources were entered in a Excel data bases for use in subsequent analyses.

3.3.10 Reporting

The following reports were prepared submitted as per schedule.
Inception Report
Mid-Term Progress Report
Draft Report: This report for comments
Final Report: prepared and submitted.

CHAPTER-4

LITERATURE REVIEW

4.1 Literature Review: Thalassaemia

4.1.1 Thalassemia in 19th Century

Von Jaksch (1889) described an anemia accompanied by splenomegaly and leucocytosis which he gave the name 'Anaemia infantum pseudoleucaemica', this was subsequently called "Jaksch-Hayem-Luzet's" anemia after the names of the authors.

4.1.2 Thalassemia in 20th Century

In 1925, Thomas Cooley, a Detroit pediatrician, described a severe type of anemia in children of Italian origin. He noted abundant nucleated red blood cells (RBCs) in the peripheral blood, which he initially thought was erythroblastic anemia, an entity that Von Jaksh (1889) described earlier. Although Cooley was aware of the genetic nature of the disorder, he failed to investigate the apparently healthy parents of the affected children. Rietti (1925) focused on an account of primary hemolytic icterus. Cooley (1927) further refined his concepts on rythroblastic anemia. Cooley et. al (1927) studied the Anemia in children with splenomegaly and peculiar changes in the bones. Whipple and Bradford (1932) reported the first complete autopsy with Cooley's anemia in which they called attention to excessive pigment deposition in many organs; they first suggested the term thalassemia. Studied the Mediterranean disease–thalassemia and associated pigment anomalies simulating hemochromatosis was studied by Whipple and Bradford (1936). Caffey (1937) had hypothesized that mild cases can reach adult life and transmit the diseases. Caminopetros (1938) pointed that the disorder was transmitted as a Mediterranean recessive. He also proposed the existence of genetic carrier, as evidence by blood studies, fragility tests and mild roentgenographic changes. Valentine and Neel (1944) coined out the term "thalassemia major" and "thalassemia minor". The presence of increased alkali resistant hemoglobin in patients with thalassemia and demonstrated increased fetal hemoglobin in thalassemia first studied by Vecchio (1946).

Sturgeon and co-workers (1952) demonstrated that patients with chronic hemolytic anemia associated with thalassemia and sickling trait. In 1955, Smith et al. his studies in Mediterranean (Cooley's anemia) I. Clinical and hemato aspects of spenectomy with special reference to fetal hemoglobin synthesis. Sturgeon et al. (1955) pointed out the chronic hemolytic anemia associated with thalassemia and sickling trait. Sturgeon, et al (1955a) Searched and described intermediate types of thalassemia clinically, genetically and biochemical studies of intermediate type of Cooley's anemia. Observations on the minor basic hemoglobin components in the blood of normal individuals and patients with thalassemia observed by Kunkel et al. (1957). The role of spenectomy in the management of thalassemia find out by Smith et al. (1960). Curtain et al. (1962) finds out the distribution pattern, population genetics and anthropological significance of thalassemia and abnormal hemoglobins in Melanesia. Thalassemia and abnormal human hemoglobin studied by Itano and Pouling (1961). Nathan and Gunn (1966) studied the consequences of unbalanced hemoglobin synthesis in thalassemia. Dauphinee and Langley (1967) studied the Thalassemia in Canadians. Kaplan et al. (1964) studied fifty cases of Cooley's anemia for dental and oral findings. Acquired hemoglobin H-disease demonstrated by Hamilton et al. (1971). Roy et al.

(1971) observed the radiological changes of bones in thalassemia syndrome. Lodish and Jacobsen (1972) in his research work he studied the regulation of hemoglobin synthesis and equal rates of translation and termination of α- and β-globin chains. Kan (1977) Identify the nondeletion defect in a-thalassemia. Parfrey et al. (1981) he observed the Iron overload in beta-thalassaemia minor.

Prevention of thalassemia in Cyprus observed by Angastiniotis and Hadjiminas (1981).Genotypic analysis of symptomatic thalasaemic syndromes observed by Agarwal and Mehta (1982).The thalassemia syndromes in India stuied by Chouhan (1983). . Mahadik et al.(1986) finds out the one tube osmotic fragility as a useful screening test for thalassemia carriers. White et al. (1986) studied the Iron state in alpha and beta thalassemia trait. Mehta and Pandya (1987) observed the Iron status of beta thalassemia carriers in India. In Greece, Fessas (1987) he pointed out the prevention of thalassemia and haemoglobin S syndromes. Phaedon (1987) studied the prevention of thalassemia and haemoglobin S syndromes in Greece. Spirito et al. (1990) studied the restrictive diastolic abnormalities identified by Doppler echocardiography in patients with thalassemia major. Sangani et al. (1990) studied thalassemia in Bombay and their role in medical genetics in developing countries. Sharma et.al. (1990) find out the Desferrioxamine use in iron chelation in thalassemia. Varaswala et al. (1991) studied the spectrum of beta thalassemia mutations in the Indian subcontinent on the basis of prenatal diagnosis.

Status of hemoglobinopathies in India studied by Marwah and Lal (1994).B-Thalassemia in Turkey studied by Ghazi Omar and Tadmouri (1994). Naveed (1995) in his research work he studied the clinical, biochemical and molecular characterization of thalassemia syndromes in Uttar Pradesh. The clinical and hematological profile of sickle cell disease cases in India find out by the author Balgir (1995). Xu et al. (1996) observed the antenatal screening and fetal diagnosis of beta-thalassemia in a Chinese population. Fuchs et al. (1996) finds the nutritional factors and thalassemia major.

Dumars et al. (1996) he written the book on practical guide to the diagnosis of thalassemia. Beta thalassemia major and successful pregnancy studied by the author Kumar et al. in 1997. Management of thalassemia induced skeletal facial deformity was observed by Drew and Sach (1997). Agarwal et al. (1997) studied the hemoglobin E-beta thalassemia in Uttar Pradesh. Choudhry et al. (1997) find out the hematological responses to hydroxyurea therapy in multi transfused thalassemic children. Low (1997) observed the hormone and growth abnormalities in untreated and treated beta thalassemia. Laopodis et al. (1998) studied the laparoscopic splenectomy in β-thalassemia major patients. Thailand studied by Kor-anantakul et al. (1998). A simple screening test for the detection of heterozygous thalassemia pointed out by Thool et al. (1998). Current Trends in Management of the beta thalassemia pointed out by Shah et al. (1999). Carrier screening and prenatal diagnosis of b-thalassemia studied by Maheswari et al. (1999).Sickle-cell and thalassemia genes in Saudi Arabia studied by El-Hazmi and Warsy (1999).

4.1.3 Thalassemia in 21st Century

In recent years, the molecular biology and genetics of the thalassemia syndromes have been described, revealing the wide range of mutations encountered in each type of thalassemia. β thalassemia alone can arise from any of more than 150 mutations. Screening for hemoglobinopathies and growing health problem in the Nordic countries observed by Birgens (2000). Maram et al., (2000) studied the effectiveness of osmotic fragility screening with varying saline concentrations in detecting thalassemia trait. Cappellini et al. (2000) finds out the venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. Krishnamurti (2000) in his report he observed the hemoglobin E-beta-thalassemia in Northeast India. Wild and Bain (2001) Investigates the abnormal hemoglobins and thalassemia. Agha (2000) in his Ph.D. thesis he evaluate maxillofacial anomalies in B-tha-lassemia major. Ahmed et al. (2000) studied the prenatal diagnosis of β- thalassaemia in Pakistan. Rachmilewitz and Schrier (2001) observed the pathophysiology of β thalassemia, disorders of hemoglobin. In 2001, WHO, UNICEF and UNU reported the assessment, prevention and control of Iron deficiency anaemia. *Geographic distribution of thalassemia observed by Bernini (2001)*. In Tehran, Tabatabei et al. (2003) observed the metabolic and endocrine complications in beta-thalassemia major. Jamal (2004) find out the burden of thalassemia in Malaysia.

Yi Kong *et al.* (2004) found that the loss of α-hemoglobin–stabilizing protein impairs erythropoiesis and exacerbates β-thalassemia. Cunningham *et al.*, (2004) studied the **c**omplications ofβ-thalassemia major in North America. Rahman and Lodhi (2004) studiedthe prospects and future of conservative management of beta thalassemia major in a developing country. Walter *et al.* (2006) studied the oxidative stress and inflammation in iron-overloaded patients with beta-thalassaemia or sickle cell disease. In 2005, Leung *et al.* has observed the Thalassemia screening in pregnancy. Human genetics programme, (WHO, 1983) reported the community control of hereditary anemia's. World Health Organization and UNICEF (2005) reported malaria in the World. Hahalis *et al.* (2005) observed the heart failure in beta thalassemia syndromes. Panigrahi *et al.* (2005) observed the cord blood analysis for prenatal diagnosis of thalassemia major and hemophilia.

Piga et al. (2006) in his study trials the randomized phase II of deferasirox (Exjade, ICL670) in comparison to deferoxamine in thalassemia patients with transfusional iron overload. Atika et al. (2006) observed the psychosocial burden in Thalassemia. Pennell et al. (2006) finds outthe controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. Abdalla (2006) observed the dental development in subjects with TM. Eshghi et al. (2007) finds the growth impairment in beta-thalassemia major: the role of trace element deficiency and other potential factors. Rajendran and Sivapathasundaram (2007) find out the diseases of blood and blood-forming organs. Cohen etal.(2008) studies the effect of transfusional iron intake on response to chelation therapy inbeta-thalassemia major. Leonardi et. al (2008) studied the relationship of magnetic resonance imaging estimation of myocardial iron to left ventricular systolic and diastolic function in thalassemia. In recent years, Pseudoxanthoma Elasticum-like Syndrome and Thalassemia find out by the author Elena et al. (2009). Thalassemic mothers and their babies studied by Somchai et al. (2009). Wood et al. (2010) studies the effect of deferasirox on cardiac iron in thalassemia major and its impact on total body iron stores. Cardiovascular MRI in thalassemia major studied by Wood and Noetzli (2010). Long-term chelation therapy with deferasirox: effects on cardiac iron overload measured by T2 MRI observed by Ruffo *et al.*(2010). Abolfazl *et al.* (2010) observed the Zinc and copper status in children with TM. Mohammad *et al.* (2010) finds out the frequency of beta thalassemia trait and carrier in Gorgan, Iran. Screening for Thalassemia and Hemoglobinopathies in Canada observed by Bijayini *et al.* (2010). The observations and study on BT was studied by Cao and Galanello (2010). James *et al.*, (2010) elevated exhaled carbon monoxide concentration in hemoglobinopathies and its relation to red blood cell transfusion therapy. Hira *et al.* (2011). Newborn Screening in India is studied by Seema Kapoor and Madhulika Kabra (2010). In 2010, Balgir found the phenotypic diversity of sickle cell disorders with special emphasis on public health genetics in India. Singh *et al.*, (2010) studied the effect of wheat grass tablets on the frequency of blood transfusions in Thalassemia MajorFind out the complications in thalassemia patients receiving blood transfusion. Thompson *etal.*, (2011) investigated the red cell alloimmunization in a diverse population of transfusedpatients with thalassemia. Recently, Abdelmohsen (2011) studied the exhaled carbon monoxide concentration in beta-thalassemia and its relation to red blood cell transfusion therapy in pediatrics. Cappellini *et al.,* (2011) observed iron chelation with deferasirox in adult and pediatric patients with thalassemia major. Recently Arıca *et al.* (2012) evaluate the hemoglobinopathy screening results of a six year period in Turkey. Fabrice et al (2012), observed the genetic modifiers of beta-thalassemia and clinical severity as assessed by age at first transfusion. Marion *et al.* (2012) studied the α^0-thalassemia deletion in a Greek patient with HbH disease and β-thalassemia trait. Yixuan *et al.* (2012) studied genetic correction of β-thalassemia patient-specific iPS cells and its use in improving hemoglobin production in irradiated SCID mice.

Source: Survey of Blood Tranfusion – induced malari and other diseases in Thalassaemia patients from Solapur (MS) district, india by miss swarupa basavraj dama.2012

4.2 Literature review: Prevalence of Thalassaemia

The prevalence of the alpha and beta globin defects have not been analyzed in all countries worldwide, primarily due to lack of resources and greater clinical priorities. However a number of workers have undertaken these studies in individual countries .Worldwide figures from the WHO suggest that there are 270 million carriers for globin gene defects and that 300,000 to 400,000 children are born annually with a severe haemoglobin disorder[1]. Recent surveys suggest that between 300,000 and 400,000 babies are born with a serious hemoglobin disorder each year and that up to 90% of these births occur in low- or middle-income countries[2]. Hemoglobin SC disease is more restricted to parts of west and north Africa whereas HbS β thalassemia occurs in localized parts of sub-Saharan Africa and sporadically throughout the Middle East and Indian subcontinent. Hemoglobin E occurs widely throughout the eastern half 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 “hemoglobin E triangle,” up to 70% of the population are carriers[3].

4.2.1 Thalassemia in the World

Thalassemia is found in some 60 countries with the highest prevalence in the Mediterranean region, parts of North and West Africa, the Middle East, the Indian subcontinent, southern Far East and southeastern Asia, together composing the so-called thalassemia belt. In western countries, thalassemia affects mostly individuals whose ancestry are traceable to a high prevalence areas.[4-7] As an example, there are around 1,000 cases of ß-thalassemia major in the United States, most of whom are descendants of Mediterranean, Asian Indian, South Asian, or Chinese ancestors.[4] The distribution of the disease, even in the thalassemia belt is not uniform. The highest frequency of the Alpha-thalassemia genes is found in Southeast Asia and among those whose ancestors settled there from the West Coast of Africa. The population of northern Thailand, with a prevalence of about 5% to 10%, harbors one of the highest incidences of Alpha -thalassemia in the world.[8] The incidence of Alpha -thalassemia carriers in Portugal is also high (10%).[9] In the eastern oases of Saudi Arabia, more than half of the people have a clinically silent form of Alpha -thalassemia.[10] With the resettlement of nearly two million refugees from Cambodia, Laos, and Vietnam in the 1970s and 80s, in the United States and Europe, symptomatic Alpha -thalassemia syndromes, although still insignificant, appear to gain more importance.[11] About 150 million people worldwide carry ß-thalassemia genes. The genes are particularly prevalent in Italy and Greece. Other regions with the high gene frequency are Sardinia (11-34%),[12] Sicily (10%),[13] Greece (5-15%),[13] and Iran (4-10%)[14]. High prevalence of both Alpha and ß-thalassemia is also present in southern China and Taiwan.[15]

A major factor for genetic diversity and thalassemia gene dissemination is the migration of people.. Carrier states are also found in virtually every ethnic group residing in the region, with higher-than-expected rates in non-Hispanic whites (1:600 births).[4] The majority of ß-thalassemia carriers in India have been migrants from Pakistan.[16] Many of ß-thalassemia gene mutations detected in Lebanon, were of Turkish, Iranian, Kurdish, Bulgarian and Asian Indian origin.[17] In Turkish Kurdistan, the primary mechanism for the development of ß-thalassemia is genetic admixture with the local population.[18]In a study carried out in Argentina, it was revealed that the diversity of ß-thalassemic alleles, together with their distribution, were similar to that found in the Mediterranean area.[19] In another more recent investigation, it has been found that ß-thalassemia in Argentina originated mainly from Italian immigrants.[20] And, in Germany, ß-thalassemia has originated from the Mediterranean region in about two-thirds of cases.[9]

4.2.2 Thalassemia in Asia

Thalassemia is very prevalent in Asia causing public health and socioeconomic problems in many countries. Alpha-thalassemia, beta-thalassemia,and hemoglobin (Hb E), are commonly noted as the hallmark of Southeast Asia, Sri Lanka, Bangladesh, Maldives and the eastern region of India.

Hemoglobin Constant Spring (Hb CS) is also prevalent in the Southeast Asia Region. Furthermore, the most serious form of thalassemia, Hemoglobin Bart's hydrops fetalis, is almost exclusively found in Southeast Asians and South China [21-24] .Large population screening examinations in Japan and Korea revealed very few thalassemia. Both alpha and beta-thalassemia are prevalent among Southern Chinese.Interaction between beta thalassemia genes or beta thalassemia and Hb E genes leads to homozygous beta thalassemia and beta thalassemia/Hb E that are major beta thalassemic syndromes in this region.In Iran, like many other countries in the region, a large number of major thalassemia patients.[14] The gene frequency of ß-thalassemia, however, is high and varies considerably from area to area, having its highest rate of more than 10% around the Caspian sea, and Persian Gulf. The prevalence of the disease in other areas is between 4% and 8%. In the Fars Province, in southern Iran, the gene frequency is also high and reaches 8-10%.[14]

4.2.3 Southeast Asia

In Southeast Asia α-thalassaemia, β-thalassaemia, haemoglobin (Hb) E and Hb Constant Spring (CS) are prevalent. The abnormal genes in different combinations lead to over 60 different thalassaemia syndromes, making Southeast Asia the locality with the most complex thalassaemia genotypes.Southeast Asia consists of 10 countries with a total population of about 400 million. The ethnic origins of people living in these countries are very heterogeneous. The Mon-Khmer and Tai language-speaking people occupy Thailand, Laos, Cambodia and some parts of Vietnam, Myanmar and Malaysia. The west includes the Burmese (Tibeto-Burman) and the northeast is the Vietnamese (Austro-Asiatic). The Malayopolynesians (Austronesian) live in Malaysia, Indonesia, Brunei, the Philippines and a number of Pacific island nations. Chinese and Indians are relatively newcomers spread throughout the region[25] . Hb E is the hallmark of Southeast Asia attaining a frequency of 50-60 per cent at the junction of Thailand, Laos, and Cambodia.The four major thalassaemic diseases are Hb Bart's hydrops fetalis (homozygous α-thalassaemia 1), homozygous β-thalassaemia, β-thalassaemia/Hb E and Hb H diseases.Compound heterozygosity between β-thalassaemia and Hb E leading to β-thalassaemia/Hb E disease is also common. The patients present variable severity of anaemia, and some can be as severe as homozygous β-thalassaemia[26]. This condition poses a major public health problem throughout Bangladesh, Burma and Southeast Asia. In Sri Lanka nearly 50 per cent of transfusion-dependent thalassaemic children were found to have β-thalassaemia/Hb E[27,28]In Thailand approximately 3000 new cases are born each year while there may be ten times as many in Indonesia.

4.2.4 Beta Thalassemia in India

Earlier studies have shown that the overall prevalence of β-thalassemia is 3–4 % with an estimate of around 8,000 to 10,000 new births with major disease each year (Madan et al. 2010)[29] ;(Balgir 1996[30]) in India. Whereas β-thalassemia is the commonest single-gene disorder in the Indian population [31] Ten percent of the total world thalassemics are born in India every year [32] . Certain communities in India, like Sindhis, Gujratis, Punjabis, and Bengalis, are more commonly affected with beta thalassemia. It has been estimated that the prevalence of pathological hemoglobinopathies in India is 1.2/1,000 live births [33], and with approximately 27 million births per year [34] this would suggest the annual birth of 32,400 babies with a serious hemoglobin disorder. Within this overall disease classification a 1989 WHO Working Group on guidelines for the control of hemoglobin disorders estimated a 3.9% carrier frequency for b-thalassemia in India, encompassing all types of b-thalassemia trait [35] . A WHO update on b-thalassemia in India indicated a similar overall carrier frequency of 3–4%, which given the current national population would translate to between 35.6 and 47.5 million carriers of the disorder nationwide [36,37] · More than 200 thalassemia mutations have been identified all over the world [38]

4.2.5 Thalassaemia in In Bangladesh

Thalassemia is the most common genetic blood disease in the world and varies in different population group in the world[39].World Health Organization (WHO) estimates that at least 6.5% of the world populations are carries of different inherited disorders of hemoglobin.[40]

Another WHO report estimates that 3% are carriers of beta-thalassemia and 4% are carriers of Hb E in Bangladesh. In Bangladesh, more than 7000 children are born with thalassemia each year.[41]
In Bangladesh, a study carried out by the Dhaka Shishu Hospital Thalassemia Center in 2004 in school children of Bangladesh, showed that carrier status is higher and there is also regional variation. This study revealed that the overall prevalence of beta-thalassemia trait in Bangladesh was 4.1% and Hb E trait 6.3%.[42] .A recent study showed that carrier status of Hb-E is 6.1% and as high as 40% in tribal children in Bangladesh.[43]

Thalassemia and other structural haemoglobinopathies are the major erythrocyte formation disorder prevalent in certain parts of the world including Bangladesh. One study(Mesbah Uddin et al)[44]investigated 600 cases of anaemic patients referred from various parts of the country for diagnosis and counselling during 3 months (April to June 2011) of time. The most common form of haemoglobin (Hb) formation disorder observed in 600 subjects studied was β-thalassemia minor (21.3%). Two other conditions, such as E-β-Thalassemia and HbE trait, were also fairly common (13.5 and 12.1%, resp.) in the total subjects studied. Other forms of haemoglobin formation disorders observed were HbE disease (9.2%), Hb D/S trait (0.7%), β-thalassemia major (0.5%), and δ-β-thalassemia (0.5%). The majority of the haemoglobinopathies belonged to neonatal to childhood period (0–15 years), followed by reproductive age group (16–45 years). Few old-age (46 + years) cases were also detected in course of clinical complications.Hemoglobin E (HbE) is an important hemoglobin variant with worldwide distribution and commonly found in Southeast Asian populations. In Bangladesh a number of these disorders have been reported in different area. But in Rajshahi (northwest region of country), there is no study on the prevalence of hemoglobin E.

A retrospective descriptive observational study was conducted period were for two years from 1 January 2009 to 31 December 2010 in Rajshahi [45]. During this period they analyzed blood samples of 537 anemic patients in the population of Rajshahi. Age ranges were 1 to 60 years. A total of 38 (or 7%) samples were found to be possessing the hemoglobin E trait.The findings of this study close to the study carriedout by Dhaka Shishu Hospital Thalassemia Center in 2004 in school children of Bangladesh showedthat the overall frequency of Hb-E trait 6.3%**1**. In Bangladesh more than 7,000 children are born with thalassemiaeach year[46].

The incidence for this disease is high in tropical and subtropical areas including Southeast Asia[47]. In Bangladesh the carrier rate of β-thalassemia is 3.0% and Hb-E/β- thalassemia is 4.0% and affected birth per thousand of β-thalassemia and Hb-E/ β-thalassemia is 0.106 & 3.000 respectively[48]About 10% of the world's thalassemia major children are born in India[49]. Gene flow from Indian sub-continent to Bangladesh may be one of the reasons of prevalence of this disease in Bangladesh[50,51].Other reasons of prevalence of thalassemia disease are intermarriage between different ethnic groups, lack of awareness for blood test before marriage [52] . Besides accurate data on carrier rates in Bangladesh is lacking, insufficient technologies for prenatal diagnosis also increases the prevalence of beta thalassemia in Bangladesh.

Therefore, from the result it can be concluded that the mutation IVS-1-5 G>C is the most common for the occurrence of β-thalassemia among the Bangladeshi population as Indian Subcontinent and South East Asia.In a study, nine mutations (-90 C>T, Codon 1 T>A, Codon 2 C>A, Codon 2 T>C, HbE /Codon 26 G>A, Codon 30 G>C, IVS-I-5 G > C, IVS-2-16 G>C and IVS-2-81 C>T) of HBB gene were found in the 70 Bangladeshi β thalassemia patients81.4%[53].

4.3 Literature Review: Characteristics of Patients

Thalassemia is a problem of 60 countries with the highest prevalence in the Mediterranean region, parts of North and West Africa, the Middle East, the Indian subcontinent, southern Far East and southeastern Asia [54, 55]**.**Beta-thalassemia represents a group of recessively inherited hemoglobin disorders first described by Cooley and Lee[56.]According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world [57

4.3.1 Beta thalassemia

Beta-Thalassemia, which is caused by a decrease in the production of b-globin chains, (affects multiple organs and is associated with considerable morbidity and mortality.[58] It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta-thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world.

4.3.1.1 Beta-thalassemia Major

The homozygous state results in severe anemia, which needs a regular blood transfusion[59,60].Clinical presentation of thalassemia major occurs between 6 and 24 months. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occour. In some developing countries, where due to the lack of resources patients are untreated or poorly transfused. In recent years, several authors reported that a high incidence of abnormalities in children, adolescents and young adults suffering from thalassemia major [61,62] Transfusion-associated viral hepatitis resulting in cirrhosis or portal hypertension also may be occurred [63,64]. In Italy 50% of thalassemic patients were estimated to have died before the age of 12 years [65]. Cornell Medical Center reported a median survival of 17.1 years in patients from 1960 to 1976[66The advent of safe transfusions has drastically prolonged the life of these patients. However, repeated transfusions have various complications and iron overload [67.

4.3.1.2 Beta-thalassemia intermedia

Individuals with thalassemia intermedia present later than thalassemia major, have milder anemia and by definition do not require or only occasionally require transfusion. At the severe end of the clinical spectrum, patients present between the ages of 2 and 6 years and although they are capable of surviving without regular blood transfusion, growth and development are retarded. At the other end of the spectrum are patients who are completely asymptomatic until adult life with only mild anemia. Hypertrophy of erythroid marrow with the possibility of extramedullary erythropoiesis, a compensatory mechanism of bone marrow to overcome chronic anemia, is common. Its consequences are characteristic deformities of the bone and face, osteoporosis with pathologic fractures of long bones and formation of erythropoietic masses that primarily affect the spleen, liver, lymph nodes, chest and spine. Enlargement of the spleen is also a consequence of its major role in clearing damaged red cells from the bloodstream.As a result of ineffective erythropoiesis and peripheral hemolysis, thalassemia intermedia patients may develop gallstones, which occur more commonly than in thalassemia major [68] .Patients with thalassemia intermedia frequently develop leg ulcers and have an increased predisposition to thrombosis as compared to thalassemia major, especially if splenectomised. Such events include deep vein thrombosis, portal vein thrombosis, stroke and pulmonary embolism [69].Although individuals with thalassemia intermedia are at risk of iron overload secondary to increased intestinal iron absorption, hypogonadism, hypothyroidism and diabetes are not common [70]. Women may have successful spontaneous pregnancies. However, if blood transfusions are necessary during pregnancy, those never or minimally transfused are at risk of developing hemolytic alloantibodies and erythrocyte autoantibodies. Intrauterine growth retardation, despite a regular transfusion regimen, has been reported[71] . Cardiac involvement in

thalassemia intermedia results mainly from a high-output state and pulmonary hypertension, while systolic left ventricle function is usually preserved [72].

4.3.1.3 Beta-thalassemia minor

Carriers of thalassemia minor are usually clinically asymptomatic but sometimes have a mild anemia. When both parents are carriers there is a 25% risk at each pregnancy of having children with homozygous thalassemia.

4.3.1.4 Hb E/beta-thalassaemia

Depending on the severity of symptoms three categories may be identified: Mild HbE/beta-thalassemia: It is observed in about 15% of all cases in Southeast Asia. This group of patients maintains Hb levels between 9 and 12 g/dl and usually does not develop clinically significant problems. No treatment is required. Moderately severe HbE/beta-thalassemia: The majority of HbE/beta-thalassemia cases fall into this category.The Hb levels remain at 6-7 g/dl and the clinical symptoms are similar to thalassemia intermedia. Transfusions are not required unless infections precipitate further anemia. Iron overload may occur. Severe HbE/beta-thalassemia: The Hb level can be as low as 4-5 g/dl. Patients in this group manifest symptoms similar to thalassemia major and are treated as thalassemia major patients.Severe HbE/beta-thalassemia: The Hb level can be as low as 4-5 g/dl.

4.3.2 Clinical Severity

- **Liver, Gall bladder, Spleen**

Abdominal examination may reveal changes in the liver, gallbladder, and spleen. Patients who have received blood transfusions may have hepatomegaly or chronic hepatitis due to iron overload [73]. Frequent blood transfusion can lead to iron overload especially in liver. Chronic iron overload may lead to cirrhosis. Transfusion- transmitted hepatitis B and C also can develop to cirrhosis. Second leading cause of death in this patient after 15 years of old is cirrhosis. In these times most of them affected with cirrhosis, hepatitis B and C [74] . A study about blood borne viruses in Mashhad on 360 thalassemic patients showed that 30 (8.33%) had a positive anti-HCV antibody, and 8 patients (2.22%) had positive HBs antigen [75].

- **Growth retardation & facial changes**

The clinical picture of thalassemia major is characterized by growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency to a mongoloid slant of the eye, and hypertrophy of the maxillae, which tends to expose the upper teeth,mucosal discoloration) It is also known as “Chipmunk face” . If a regular transfusion program that maintains a minimum Hb concentration of 9.5 to 10.5 g/dL is initiated, growth and development tends to be normal up to 10 to 12 years [57]Bimaxillary protrusion and other abnormalities are also frequently seen in thalassemia major cases (Salehi et al.,2007). A local study by Hospital University Kebangsaan Malaysia found a prevalence of short stature of 54.5% for transfusion dependent Thalassaemics (Hamidah et al., 2001). Truncal shortening was also seen in the majority of patients with short stature (Rodda et al., 1995; Hamidah et al., 2001). Recombinant growth hormone therapy has been found to have a role in the treatment of short stature in Thalassaemics (Cavallo et al., 2001; Theodoridis et al., 1998). A study in Thailand also found that adequate iron chelation and hormonal treatment enabled patients to achieve normal adult height (Viprakasit et al., 2001).

- **Hematologic changes**

RBC indices show microcytic anemia. Thalassemia major is characterized by reduced Hb level (<7 g/dl), mean corpuscolar volume (MCV) > 50 < 70 fl and mean corpuscolar Hb (MCH) > 12< 20 pg. Thalassemia intermedia is characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor is characterized by reduced MCV and MCH, with increased Hb A^2 level [76].

- **Complications of Thalasssemia due to iron over load**

Transfused patients may develop complications related to iron overload. Complications of iron overload in children include growth retardation and failure or delay of sexual maturation. Later iron overload-related complications include involvement of the heart (dilated myocardiopathy or rarely arrythmias), liver (fibrosis and cirrhosis), and endocrine glands (diabetes mellitus, hypogonadism and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands) [77]. Other complications are hypersplenism, chronic hepatitis (resulting from infection with viruses that cause hepatitis B and/or C), HIV infection, venous thrombosis, and osteoporosis. The risk for hepatocellular carcinoma is increased in patients with liver viral infection and iron overload [78]. Individuals who have not been regularly transfused usually die before the second-third decade. Survival of individuals who have been regularly transfused and treated with appropriate chelation extends beyond age of 40 years. In fact, cardiac complications are the cause of the deaths in 71% of the patients with beta-thalassemia major [79]. Blood transfusions are important for patients with anemia, chronic transfusions inevitably lead to ironoverload as humans cannot actively remove excess iron. The cumulative effects of iron overload lead to significant morbidity and mortality, if untreated (80) . A unit of red blood cells transfused contains approximately 250 mg of iron (81) while the body cannot excrete more than 1 mg of iron per day. A patient who receives 25 units per year, accumulates 5 grams of iron per year in the absence of chelation(82). Prevalence studies found that the common infections occurring in Thalassaemic patients are Hepatitis C (2.2 – 44%) followed by Hepatitis B (1.2 – 7.4 %) and HIV (0-9%) (Al-Sheyyab et al., 2001; Moatter et al., 1999; Sur et al., 1998; Laosombat et al., 1997.

- **Cardiac complications**

Cardiac diseaseis the major cause of death in thalassemia patients with iron overload. : In the mid-1960s, only 37% of patients in a small group of 41 patients with thalassemia major were alive at the age of 16 years.[83] At the beginning of the new millennium, survival at the age of 35 years was 50% according to the UK thalassemia registry[84] and 65% according to an Italian study,[85] .Most of the cardiac complications found in Thalassaemia patients were heart failure (5.4-12%) (Kremastinos et al., 2001; Aessopos et al., 2001; Fucharoen et al., 2000; Borgna-Pignatti, 1998; Kremastinis et al., 1995;), heart disease/cardiac impairment (37-51%) (Sau et al., 1995; Richardson et al., 1993; Wolfe et al., 1985), arrhythmias (5%) (Borgna-Pignatti, 1998), pulmonary hypertension (59.1%) (Aessopos et al., 2001) and diastolic dysfunction (50-54%) (Vaccari et al., 2002; Yaprak et al., 1998;Hou et al., 1994). There is a significant correlation between serum ferritin levels and myocardial iron grade and content (Lombardo et al., 1995). A study found a higher incidence of heart problems in those with poor iron chelation compliance (Wolfe et al., 1985). Another study found that the risk factors of heart disease includes old age, late age of commencement of Desferrioxamine, higher liver iron and higher serum ferritin levels (Richardson et al., 1993). Cardiovascular T2-star (T2*) magnetic resonance is useful in detecting early myocardial overload (Anderson et al., 2001). It was found that mean survival of those not on chelation was 17.4 years versus 31 years for those who received hypertransfusion and chelation (Ehlers et al., 1991). Another study states that survival after a heart failure episode was 48% (Kremastinos et al., 2001).

- **Pubertal delay**

There is a high prevalence rate of pubertal delay, ranging from 38-90% in both sexes (Filosa et al., 2001; Soliman et al., 1999; Saka et al., 1995; Kwan et al., 1995; Yesilipek et al., 1993; Borgna-Pignatti et al., 1985). A local study found that 24% of thalassaemics had delayed puberty (Jamal et al, unpublished data). The development of secondary sexual characteristics in thalassaemic children was found to be markedly delayed compared to their non-thalassaemic siblings (George et al., 1997). It was also found that there is 74% primary amenorrhoea (Soliman et al., 1999).Hypogonadotrophic hypogonadism has been found to be the main cause of delayed/failed puberty (Soliman et al., 2000).

Osteoporosis

The prevalence of osteopenia/osteoporosis amongst patients with Thalassaemia ranged from 39-67% (Chan et al., 2002; Bielinski et al., 2001; Jensen et al., 1998; Katz et al., 1994; Giuzio et al., 1991). A local study found a prevalence of osteoporosis of 84% in transfusion dependent Thalassaemics (Jamal et al, unpublished data).). Lumbar level osteoporosis is observed in treated Thalassaemic patients whilst it involves both the lumbar and femoral neck regions in those who are untreated (Lasco et al., 2001).

Diabetes Mellitus in patients with Thalassaemia

The prevalence of diabetes range from 5.4-19.5% (Chern et al., 2001; Gulati et al., 2000; Borgna-Pignatti et al., 1998; el-Hazmi, 1994), whilst impaired glucose tolerance is seen in 7.9-86% (Chern et al., 2001; Gulati et al., 2000; Zuppinger et al., 1979). It was found that most complications could be avoided if serum ferritin levels were less than1500 µg/L (Telfer et al., 2000). The frequency of diabetes in adult patients has been seen to be significantly increased by HCV infection (Labropoulou-Karatza et al., 2000).

Psychosocial effect: Thalassemia imposes a significant intrusion in the lives of patients and their families. The effects are many,sweeping from financial hard ships and absence from school and work to significant problems wih self image esteem. All of theses issues have tremendous impact of the effectiveness of therapy and on the quqlity of patients.

BT is chronic in nature and requires costly life long care and management strategies ; they cause significant health care and psychosocial burdens on the patient, the family, the health care system and the community (Weatherall and Clegg,1981: model et al.2000).Over the last 20 years, management of thalassemia major has improved to the point where patients' life expectancy will reach that of the normal population[86] .These outcomes result from safer blood transfusions, the availability of three iron chelators, new imaging techniques that allow specific organ assessment of the degree of iron overload [(86.

4.3.3 Management of Thalassemia Major Patient

- **Blood Transfusions**

The goals of transfusion therapy are correction of anemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption, which occurs in non transfused patients as a consequence of increased, although ineffective, erythropoiesis. The decision to start transfusion in patients with confirmed diagnosis of thalassemia should be based on the presence of severe anemia and to maintain Hb above 10gm% is the mainstay of treatment.

In severe anemia : (Hb < 7 g/dl for more than two weeks, excluding other contributory causes such as infection). However, also in patients with Hb > 7 g/dl, other factors should be considered, including facial changes, poor growth, evidence of bony expansion and increasing splenomegaly. Transfusions should be given in an outpatient setting and in a thalassemia care centre which has

medical staff trained to care for these patients. This is beneficial to the patients as they meet other patients with similar illness, leading to better psychological acceptance of the disease and its treatment. When possible, the decision to start regular transfusions should not be delayed until after the second- third year, due to the risk of developing multiple red cell antibodies and subsequent difficulty in finding suitable blood donors. Several different transfusional regimens have been proposed over the years, but the most widely accepted aims at a pre-transfusional Hb level of 9 to 10 g/dl and a post-transfusion level of 13 to 14 g/dl. This prevents growth impairment, organ damage and bone deformities, allowing normal activity and quality of life [87,88]. The frequency of transfusion is usually every two to four weeks. The amount of blood to be transfused depends on several factors including weight of the patient, target increase in Hb level and hematocrit of blood unit. Appropriate graphs and formulae to calculate the amount of blood to be transfused are available [87]. In general, the amount of transfused RBC should not exceed 15 to 20 ml/kg/day, infused at a maximum rate of 5 ml/kg/hour, to avoid a fast increase in blood volume. To monitor the effectiveness of transfusion therapy, some indices should be recorded at each transfusion, such as pre- and post-transfusion Hb, amount and hematocrit of the blood unit, daily Hb fall and transfusional interval.Dedicated computerized programs (Webthal) are available to monitor transfused thalassemia patients accurately [89]. Although red cell transfusions are lifesavers for patients with thalassemia, they are responsible for a series of complications and expose the patients to a variety of risks. Iron overload is the most relevant complication associated with transfusion therapy. It has been recorded that the risk of transmission of HIV, HCV, HBV from an infected donation transfused to a recipient is well over 90%. According to World health Organization (WHO) 10% of all HIV infections in developing countries is a result of transfusion with infected blood products (NACA, 2008). Sometransfusion-dependent complications arise during transfusion those are - Autoimmune hemolytic anemia, Acute hemolytic reactions.Non-Hemolytic reactions- Allergic and anaphylactic reactions, febrile non-hemolytic reactions, Circulatory overload, Post-transfusion purpura.

- **Assessment and treatment of Iron overload (Iron Chelation Therapy)**

The hemoglobin in red blood cells is an iron-rich protein; regular blood transfusions increase iron concentration in the blood. This condition is called iron overload, it damages the liver, heart, and other parts of the body. Patients maintained on a regular transfusion regimen progressively develop clinical manifestations of iron overload: hypogonadism (35-55% of the patients), hypothyroidism (9-11%), hypoparathyroidism (4%), diabetes (6-10%), liver fibrosis, and heart dysfunction (33%) [90,91]. For preventing this damage,iron chelation therapy is needed to remove excess iron from the body. To remove excess iron, patients undergo 'Iron chelation therapy,' in which a drug is introduced into the body which binds with excess iron and removes it through the urine or stool. The iron status of multi transfused patients can be assessed by several methods. Serum ferritin has in general been found to correlate with body iron stores [92] .Determination of liver iron concentration in a liver biopsy specimen shows a high correlation with total body iron accumulation and is considered the gold standard for the evaluation of iron overload [93]. In recent years, nuclear magnetic resonance imaging (MRI) techniques for assessing iron loading in the liver and heart have been introduced [94,95,96,97]. R2 and T2* parameters have been validated for liver iron concentration. Cardiac T2* is reproducible, transferable between different scanners, correlates with cardiac function, and relates to tissue iron concentration. Clinical utility of T2* in monitoring patients with siderotic cardiomyopathy has been demonstrated[98,99].Magnetic biosusceptometry (SQUID), is another option for a reliable measurement of hepatic iron concentration [100]; however, magnetic susceptometry is presently available only in a limited number of centers worldwide.

- **Iron Chelating agent**

As the body has no effective means for removing iron, the only way to remove excess iron is to use iron binders (chelators), which allow iron excretion through the urine and/or stool. As a general rule,

patients should start iron chelation treatment once they have had 10-20 transfusions or when ferritin levels rise above 1000 ng/ml [87]

- **Desferal:**

The first drug available for treatment of iron overload was *deferoxamine* (DFO)(Desferal) and for many years, the only FDA-approved iron chelator was Desferal, an exadentate iron chelator that is not orally absorbed and thus needs parenteral administration and is painful and difficult infusion process usually as a subcutaneous 8- to 12-hour nightly infusion, 5-7 nights a week. Average dosage is 20-40 mg/kg body weight for children and 30-50 mg/kg body weight for adults [87,88.] When using Desferal, a needle is attached to a small battery operated infusion pump and worn under the skin of the stomach or legs five to seven times a week for up to twelve hours (Cooley's Anemia Foundation, 2010). The only treatment options for removing excess iron were chelation (Vichinsky, 2001). With DFO, iron is excreted both in faeces (about 40%) and in urine.

Side effectOcular toxicity (night-blindness, blurred vision, decreased visual acuity, impairment of colour vision, cataract and other disturbances of the eye).Growth retarded and skeletal changes with a disproportionately short trunk and dysplasia of the long bones.Infections by *Yersinia Enterocolitica*, and other pathogens (*Klebsiella Pneumoniae*). local reactions at the site of infusion, such as pain, swelling.

The use of DFO decreases morbidity and mortality among those who are able to comply with regular prolonged infusions [101]

- **Deferiprone(DFP*)***

It is an orally active iron chelator which has emerged from an extensive search for new treatment of iron overload. Comparative studies have shown that this chelator, at doses of 75-100 mg/kg/day may be as effective as DFO in removing body iron [102.] Retrospective and prospective studies have shown that DFP monotherapy is significantly more effective than deferoxamine in decreasing myocardial siderosis in thalassemia major [103, 104, 105.]

Side effect

More common but less severe side effects are gastrointestinal symptoms, arthralgia, zinc deficiency, and fluctuating liver enzymes. Retrospective studies have shown that DFP treatment is associated with reduced cardiac morbidity and mortality [104, 106, 107.] DFO and DFP can be used in combination to achieve levels of iron excretion that cannot be achieved by either drug alone without increasing toxicity[108,109,110,111,112,113.] Reversal of severe iron-related heart failure with DFO and DFP combination has been reported in many patients [98,114,115,116,]

- ***Deferasirox* (DFX)**

is a once-daily, orally administered iron chelator that a large program of clinical trials has shown to be effective in adults and children [[117,118.] The recommended starting dose of DFX for most patients is 20 mg/kg/day, although this can be modified to 10 or 30 mg/kg/day depending on the number of transfusions a patient is receiving and whether the therapeutic goal is to decrease or maintain body iron levels.

***Side effect*:** Mild-to-moderate gastrointestinal disturbances and skin rash. These events rarely require drug discontinuation and most resolve spontaneously. Mild, usually non progressive increases in serum creatinine (generally within the upper limit of normal) has been observed in approximately a third of patients. Creatinine levels returned spontaneously to baseline in most of patients and data from up to 3.5 years of treatment in more than 1000 patients have confirmed that creatinine increase is non progressive [119.] However, cases of renal failure have been reported following the post marketing use of DFX [120.]

Treatment of iron overload-related complications

Growth deficiency: Some studies showed that the secretion of growth hormone (GH) in patients with thalassemia major have yielded contradictory results, limiting the therapeutic use of GH to those patients proven to have GH deficiency, who may have a satisfactory response to treatment [121,122,123]. In cases with signs of bone toxicity from DFO a reduction of the dose, or its substitution with an oral chelator, can prevent progression of bone lesions and improve growth.

Delayed puberty, hypogonadism and assisted reproduction

In female: For delayed puberty in girls, therapy may start with the administration of ethinyl estradiol (2.5-5 μg daily) for 6 months, followed by hormonal reassessment. If spontaneous puberty does not occur within 6 months, ethinyl estradiol should be used at increasing dosages (from 5-10 μg daily) for 12 months. If breakthrough uterine bleeding does not occur, a low oestrogenprogesterone hormone replacement is recommended. Pregnant patients with thalassemia need a multidisciplinary approach involving all specialists in the medical care of thalassemia [125].

In male: For delayed puberty in males, intramuscular depot-testosterone esters at a dose of 50-100 mg twice a month should be given, until complete virilisation has been achieved Topical testosterone gel can also be used [124]. When there is a lack of pubertal progression over a year or longer (arrested puberty), testosterone esters in males and oestrogenprogesterone replacement therapy in females is indicated.

Hypothyroidism

Preclinical hypothyroidism is characterized by normal thyroxine (T4) and free thyroxine (FT4), normal basal TSH and TSH slightly increased after the Thyrotropin-releasing Hormone (TRH) test. Therapy can be recommended for patients with TSH levels greater than 10 U/ml, thyroid abnormalities, and vague symptoms attributable to hypothyroidism. In overt hypothyroidism, characterized by low T4 and FT4 values with signs and symptoms such as mental and physical sluggishness, weight gain, feeling of cold, sleepiness, bradycardia and constipation, treatment with increasing doses of L-thyroxine starting with 25 mg per day is indicated. Abnormal thyroid function may be reversible at an early stage through intensive combined chelation [126].

Diabetes and impaired glucose tolerance

Acarbose at the dose of 100 mg (orally with breakfast, lunch and evening meals) has been used with good results for impaired glucose tolerance or non-insulin dependent diabetes mellitus and hyperinsulinism [127]. Patients with diabetes mellitus, may require daily subcutaneous injections of insulin. Investigation of the kidney function and imaging of the fundi should be carried out to evaluate the presence and degree of diabetic complications. Intensive iron chelation therapy with DFO and DFP seems to be associated with an improvement in glucose intolerance in terms of glucose and insulin secretion, particularly in patients in early stages of glucose intolerance [128].

Osteoporosis

Osteoporosis is a progressive disease; prevention is the basis of the management. Calcium-rich diet, correction of hypogonadism by sex hormone replacement therapy and regular exercise should be recommended. Oral calcium supplements should be used with caution because of the risk of renal stones. To date, alendronate, pamidronate, and zoledronate seem to be effective in increasing bone mineral density and normalizing bone turnover, but more controlled trials are necessary to evaluate their efficacy in reducing fracture risks in larger thalassemic populations [129]

Splenectomy

When annual red cell requirement exceeds 180-200 ml/Kg of RBC (assuming that the Hct of the unit of red cells is about 75%), splenectomy should be considered, provided that other reasons for increased consumption, such as hemolytic reactions, have been excluded. Other indications for splenectomy are symptoms of splenic enlargement, leukopenia and/or thrombocytopenia and increasing iron overload despite good chelation[87]. The patient should be immunized with pneumococcal, meningococcal and H influenza vaccines at least 2-4 weeks prior to splenectomy. Oral penicillin 250 mg once daily should be given for at least 5 years post splenectomy. Even minor infections should be treated with antibiotics promptly in a splenectomized patient and he should be hospitalized if fever does not subside within 48-72 hours (Shah, 2004).

Bone marrow and cord blood transplantation

Bone marrow transplantation (BMT) remains the only definitive cure currently available for patients with thalassemia. The outcome of BMT is related to the pre transplantation clinical conditions, specifically the presence of hepatomegaly, extent of liver fibrosis, history of regular chelation and hence severity of iron accumulation. In patients without the above risk factors, stem cell transplantation from an HLA identical sibling has a disease-free survival rate over 90%[130]. The major limitation of allogenic BMT is the lack of an HLA-identical sibling donor for the majority of affected patients. Provided that selection of the donor is based on stringent criteria of HLA compatibility and that individuals have limited iron overload, results are comparable to those obtained when the donor is a compatible sib [131].

Cord blood transplantation from a related donor offers a good probability of a successful cure and is associated with a low risk of GVHD [132,133]. For couples who have already had a child with thalassemia and who under take prenatal diagnosis in a subsequent pregnancy, prenatal identification of HLA compatibility between the affected child and an unaffected fetus allows collection of placental blood at delivery and the option of cord blood transplantation to cure the affected child [134].

Gene therapy

It is being tried by replacing the defective globin gene with a normal functional gene but it is technically difficult and not yet available as a therapeutic option.

Folic acid supplements

Folic acid is a B vitamin that helps build healthy red blood cells. The patient needs to take folic acid supplements in addition to blood transfusions and/or iron chelation therapy. Insufficient folic acid can aggravate the anaemia in thalassemia. Folic acid is found naturally in food such as meat and green vegetables. Patients with thalassemia who remain untransfused or are on low transfusion regimens have increased folate consumption and may develop a relative folate deficiency. Supplements (1 mg/day) may be given if this occurs. Folic acid (5 mg per week) should be given to patients receiving no or irregular transfusions,this is because of relative folate deficiency due to increased folate consumption. However,patients receiving regular blood transfusions ordinarily do not require folic acid unless actual deficiency state exists.

Management of Thalassemia Intermedia

Treatment of individuals with thalassemia intermedia is symptomatic [88,135]. As hypersplenism may cause worsening anemia, retarded growth and mechanical disturbance from the large spleen, splenectomy is a relevant aspect of the management of thalassemia intermedia. Risks associated with splenectomy include an increased susceptibility to infections mainly from encapsulated bacteria (*Streptococcus Pneumoniae, Haemophilus Influenzae and Neisseria Meningitidis*) and an increase

in thromboembolic events. Prevention of post-splenectomy sepsis includes immunization against the above mentioned bacteria and antibiotic prophylaxis as well as early antibiotic treatment for fever and malaise. Because of the elevated prevalence of cholelithiasis and the risks of cholecystitis in splenectomised patients, the gallbladder should be inspected during splenectomy and removed in case with or to prevent gallstones. If leg ulcer has developed, it is very difficult to manage. Regular blood transfusions, zinc supplementation and pentoxifylline have been proposed for ulcer treatment.

When individuals with thalassemia intermedia may develop iron overload from increased gastrointestinal absorption of iron or from occasional transfusions, chelation therapy is started when the serum ferritin concentration exceeds 300 ng/ml or when iron overload is demonstrated by direct or indirect methods [136]. Supplementary folic acid can be prescribed to patients with thalassemia intermedia to prevent deficiency from hyperactive bone marrow.

Diet for beta-thalassemia patient : Nutritional stunting as the result of reduced nutrient intake is an important cause of growth failure in young children with thalassemia and is responsive to nutritional support in thalassemic children (Fuchsa et al. 1997.However, there is no evidence that iron-poor diets are useful in thalassemia major. Only foods very rich in iron (such as liver, many baby foods, breakfast cereals and multivitamin preparations contain added iron, along with other vitamin supplements) should be avoided. Iron should not be given, and foods rich in iron should be avoided. Since many factors in thalassemia promote calcium depletion, a diet containing adequate calcium (e.g. milk, cheese, dairy products and kale) is always recommended (Galanello and Origa, 2010). The citrus fruits, guava, amla, etc. which are rich in vitamin C should be avoided. Food with high vitamin C content should preferably be taken after cooking to reduce vitamin C content. A normal diet is recommended, with emphasis on the following supplements: folic acid, small doses of ascorbic acid (vitamin C), and alpha-tocopherol (vitamin E). Drinking coffee or tea has been shown to help decrease absorption of iron in the gut.

Prognosis:

Prognosis of thalassemia minor subjects is excellent. An increased risk for cholelithiasis, especially in association with the Gilbert mutation has been demonstrated[137] Patients with thalassemia intermedia who do not usually have severe hemosiderosis are less prone to cardiac problems[138]. However, pulmonary hypertension, thromboembolic complications, overwhelming postsplenectomy sepsis, hepatocarcinoma may reduce survival in this group of patients The first advance in treatment was the initiation of episodic blood transfusions when the patient was having a particularly bad time. With the advent of this type of therapy, survival was prolonged into the second decade, but it soon became evident that the treatment that saved lives in children caused death from cardiac disease in adolescence or early childhood. Prognosis for individuals with betathalassemia major has dramatically improved with the advent of DFO. However, many transfusion-dependent patients continued to develop progressive accumulation of iron. This can lead to tissue damage and eventually death, particularly from cardiac disease. Advances in red cell transfusion, and the introduction of new iron chelators and chelation regimes have further prolonged survival in recent years.Assessment of myocardial siderosis and monitoring of cardiac function combined with intensification of iron chelation have converted a once universally fatal disease to a chronic illness and an excellent long-term prognosis is expected for children who have been chelated since a very young age [139,140].Bone marrow transplantation is at present the only available definitive cure for patients with thalassemia major.

The Compliance Problem

This can be achieved by increasing awareness about thalassemia,by screening siblings and parents of the patient to identify carriers of the disease, screening the communities in which thalassemia is very common, screening the couple before they plan to have a baby and prenatal diagnosis if the

woman is pregnant i.e. testing the fetus for thalassemia major and aborting it if found to have the disease.

4.3.4 Social Situation of Thalassaemia Patients:

Introduction:

Repeated, long and unpleasant treatments in thalassemia cause this disease cover all aspects of the individuals' life. It has severe and considerable consequences on general and mental health and quality of life of the patients and their families[141]. Most of the families can successfully adopt themselves with chronic disease of the children. In contrast, some of them also may not be successful in coping with it due to lack of access to accurate information about the disease, lack of appropriate support resources, high treatment costs, mental status and social damages[143] .Significant advances have been made over the years on thalassaemia leading to better understanding of genetic control of haemoglobin, the abnormalities in the different forms, the pathophysiology of the disease and hence improvement in treatment[142].Thalassaemia poses challenges to patients and family members at the physical, emotional and cognitive levels leading to disruption of their normal psychosocial life.

(i). Education:

Majority of the parents were concerned about their thalassaemic children leaving home to further their studies in colleges or higher institutions.The patients themselves if given the opportunity would have liked to be able to leave home to pursueeducation, but were discouraged by parents due to concerns over health. Both patients and parents believed that the disease didinfluence the academic performance. Academic performance and presence is less well mostly as a result of having to be away from school for follow-up visits, blood transfusion in the day-care centre or admission to the ward.Such absence seemed to have an impact on the academic achievement. In addition many parents attributed the poor academic performance of patients to tiredness and lethargy.It is generally agreed that the schools as well as teachers are supportive of thalassaemic children. Due to tiredness and lethargy most of the patients are not fully involved in sports or co-curriculum activities.

(ii).Psychological impact on psychical deformities:

Short Stature/Growth Failure:

Not attaining the average height compared to their friends or same age group is perceived as a major problem by both parents and patients.Patients are being often teased by their friends for their shorter stature. They are alsoconstantly being compared to other siblings at home about their height and thus not respected by younger siblings. As result of these perceived issues they felt anxious, stressed-up and emotionally upset.

Thalassaemic Facies, Skin Colour, Protrusion of Teeth:

Patients felt a degree of stigmatisation as a result of their"dark" skin colour. The thalassaemic facies also contributed significantly to the negative body image. Many of them are teased for their thalassemic faces and yellowish protruded teeth .

Delayed Menses

The parents of female adolescent patients are very concerned about their children not attaining or delayed menarche as this may influence their ability to have a family of their own in future.

(iii). Employment

The difficulty of obtaining a job was expressed mainly by patients who had studied up to only secondary school.Patients who were working admitted to having difficult relationship with their employers as they had to take time off work frequently for transfusion and other treatment.

(iv). Marriage and Starting a Family

Even though the patients were interested in looking for a lifepartner, they could not help but express their worry aboutbeing rejected by partners because of their illness. All patientsagreed however that they should be truthful to their potentialspouse about their illness.

(v) . Financial Constraint

Many parents thought that regular treatment of thalssemia / illness is a financial burden for them. For some parents financial difficulty was the expenditure incurred when going to hospital for follow up visits. Also for some of them any loss of working days meant financial loss as well.

(vi). Social Integration and Relationship

Although patients had good relationship with siblings and parents, a few problems were highlighted. Many parents claimed that their children did not have many friends and were virtually housebound.They believed that generally it was the normal children that did not want to befriend the patients. Patients attributed the illness as an obstacle for them to integrate socially.Parents of adolescent/young adult patients commented that most of their thalassaemic children were not keen on discussing about their disease. The patients themselves felt that discussing about the illness would notchange their predicament. Most patients have poor self-esteem and are emotionally affected. They have mixed feelings namely sadness and anger. Some parents reported that their children preferred not to disclose their illness to others as they were embarrassed.

(vii) . Fear Related to Complications of Blood Transfusion

Parents constantly worried about the adverse effects related totransfusion. Some of their concerns were that they are afraid if something happens during the blood transfusion.They are scared because the risk of AIDS, hepatitis B, C, either blood is safe or not, transfusion reaction.Adolescence patients indicated that they were bored with having to adhere to the long-term treatment and in particular the innumerable needle pricks.

(viii). Compliance of Subcutaneous Iron Chelation Therapy and Oral Medication

Generally compliance to subcutaneous iron chelation therapy is poor. This is due to several factors such as the side effects, simply not wanting to administer, feeling of difficulty and burdensome. Compliance with oral medication was variable though generally better than that of subcutaneous iron chelator.

The most significant finding was about the issue of psychosocial problems of the thalassaemics patients and their family members. It illustrated how ‘clinical burden’ of a disease like thalassaemia immensely affected the psychosocial aspects of families.Few similar studies have been conducted in Malaysia and as such only limited comparison could be made. Ratip et al.found similar problems among thalassaemia intermediapatients[144]. Khurana et al.and Gharaibeh H et al.also showed similar areas of concern in transfusion dependent beta thalassaemia major patients[145,146]. Many parents perceived the anaemic symptoms as another factor that contributed significantly to the poor academic performance in their thalassaemic children. This therefore aises the question about the pre-transfusion haemoglobinlevel of these children and hence compliance with follow upvisits.

The evidences indicated that mean of anxiety, depression, aggression and shyness is higher in thalassemic patients than healthy children[147]. Moreover, another study indicated that anxiety and depression were in 47% of the patients and this brought about problems in their self-care and quality of life[148] .Among the health team members, the nurses have the closest relationship with patients and their families, they would be able to use appropriate methods and cause promotion in the quality of life and finally improve life of these patients[149] .On the other hand, family which plays the main role in ensuring health and welfare of the children can have a significant impact on their quality

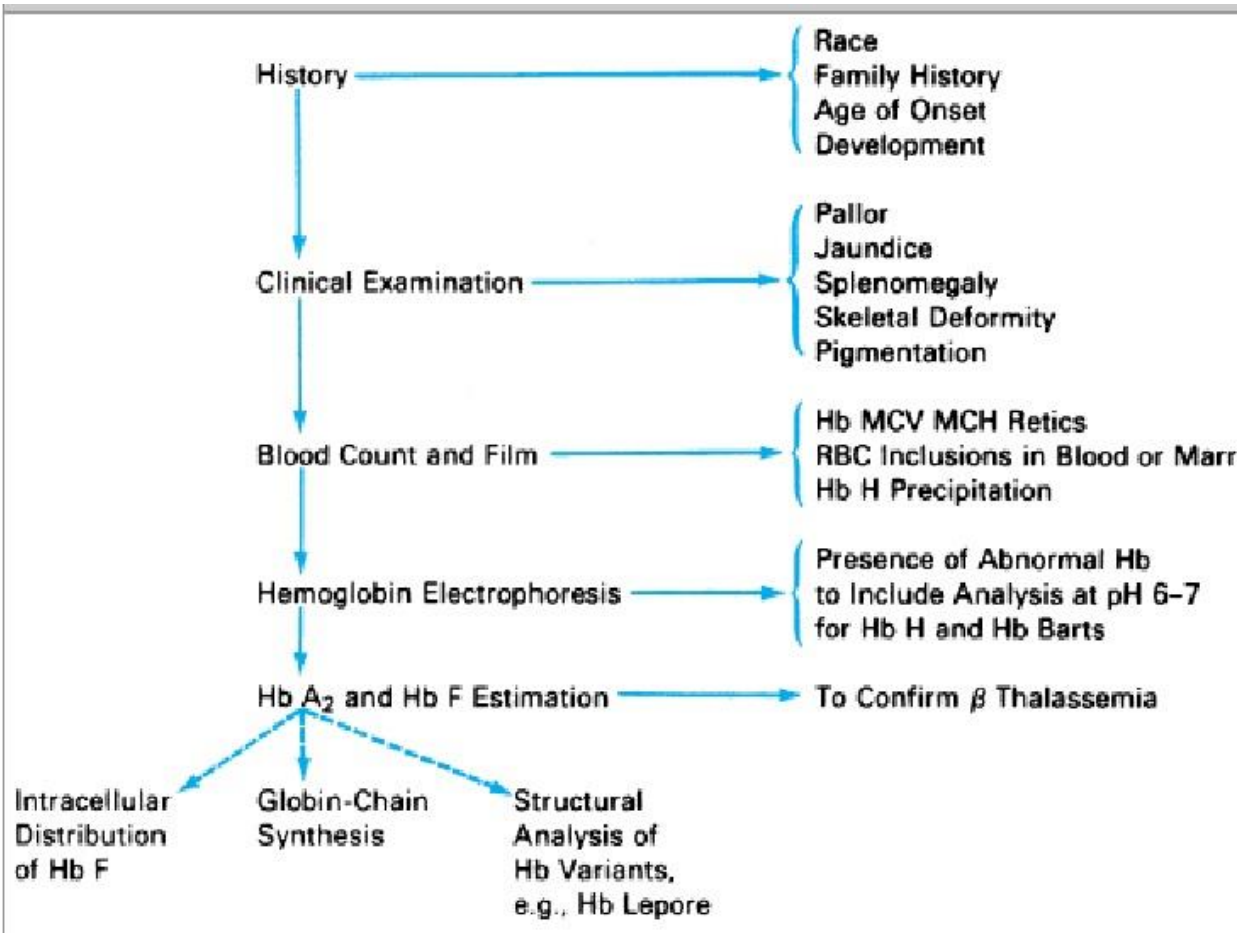
of life. That is why nowadays attention to health-care services has changed from traditional child-centered to family-centered[150].

4.4 Literature Review: Tools used for Thalassaemia Detection

4.4.1 Introduction

The aim of screening for thalassaemia and Hb disorders is to offer carrier testing to every member of the population, ideally before they have children, in order to identify carrier couples and inform them of the risk and their options.The people targeted by screening are therefore carriers of α^0-thalassaemia, β-thalassaemia and Hb E.As a general guideline, primary screening for all forms of thalassaemia involves using an electronic blood-cell counter to provide accurate erythrocyte indices. Individuals who have hypochromic microcytosis with mean corpuscular volume (MCV) below 80 fl or mean corpuscular Hb (MCH) below 27 pg should be investigated further using Hb electrophoresis or high performance liquid chromatography (HPLC)[163,164.] This, however, can be problematic in rural areas in south-east Asia where the expense usually precludes the possibility of electronic blood-cell counting. The initial approach to apprehend a suspect is shown diagrametically at Figure 4.1.

Fig 4.1: Initial Approach to Suspect



When to Screen

Screening can be targeted at different age groups. Newborn screening for β-thalassaemia is less frequent, as it requires expensive DNA analysis.

Newborn
Adolescence
Premarital
Preconceptional
Antenatal

Lab Investigations:

1. Screening tests
2. Diagnostic tests
3. Prognostic tests.

Screening tests:

- Haematological Methods :
    A. Red blood cell indices
    B. Red blood cell morphology
    C. Single tube osmotic fragility test (OFT)/ NESTROFT
    D. HbE detection by DCIP Test

Heamoglobin Pattern Analysis:

1. For HbA_2 Determination (chromatographic Methods)-
    a) HPLC for heamoglobinopathies screening
2. Electrophoretic Methods :
    a) Capillary Electrophoresis for haemoglobinopathies screening
    b) Cellulose Acetate electrophoresis
    c) Agar gel electrophoresis
    d) Isoelectric Focusing (IEF)
3. Globin chain synthesis :
    a) Weatherall and Clegg Method

Foetal sampling for the prenatal management of haemoglobinopathies

- Chorionic villi sampling (CVS) – 11th week
- Amniocentesis – 16th week
- Foetal blood sampling – 16th week

Diagnostic tests

- Electrophoresis
- HPLC

Prognostic Test:

- Biochemical parameter
  A. Serum feretinin (microgram/l)
  B. Hb(g/l)
  C. Serum iron (micromol/l)
  D. Serum TIBC(micromol/l)
  E. TISP (%)
  F. Transferrin concentratiion (mg/l)

4.4.2 Haematological methods to identify Thalassaemia Carrier and Disease:

Haematological methods contribute the basis of identification of carrier of the thalassaemias. Red cell indices and morphology, HbA2 quantification and Hb fraction separation are all used. These are methods often used as economical screening methods or as elements in the description of the carrier status.

4.4.2.1 Red Blood Cell indices

Red blood cell (RBC) indices determination is the most common laboratory test and is usually carried out by automated electronic cell counters. These counters produce many parameters of which only a few, such as the mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH) and haemoglobin (Hb) concentration, are strictly relevant and useful for haemoglobinopathies screening.

Interpretation of result: MCV and MCH are variably reduced in thalassaemia carriers. MCH ismore reliable than MCV, since the MCV does not remain stable due to a tendency for the red cells to increase in size over time. The most widely used cut-off values of MCV and MCH for indicating thalassaemia are79 fl and 27 pg, respectively. Values below these may indicate α- or β-thalassaemia or iron deficiency anemia. For β-thalassaemia, the degree of microcytosis and hypochromia as reflected by the lower than normal MCV and MCH is relatedto the severity of the underlying mutation.

Cost: Tk. 100 per test
Availablity; widely available in Bangladesh

4.4.2.2.Red blood cell morphology

Morphological changes of red cells can be detected in most thalassaemia carriers. An examination of a stained peripheral blood smear may be helpful in the evaluation of cases.

Preparation of smears: EDTA anticoagulated venous whole blood or capillary blood is used to prepare a blood film. After drying at room temperature the blood film can be stained with May-Grumwald (or Wright stain)

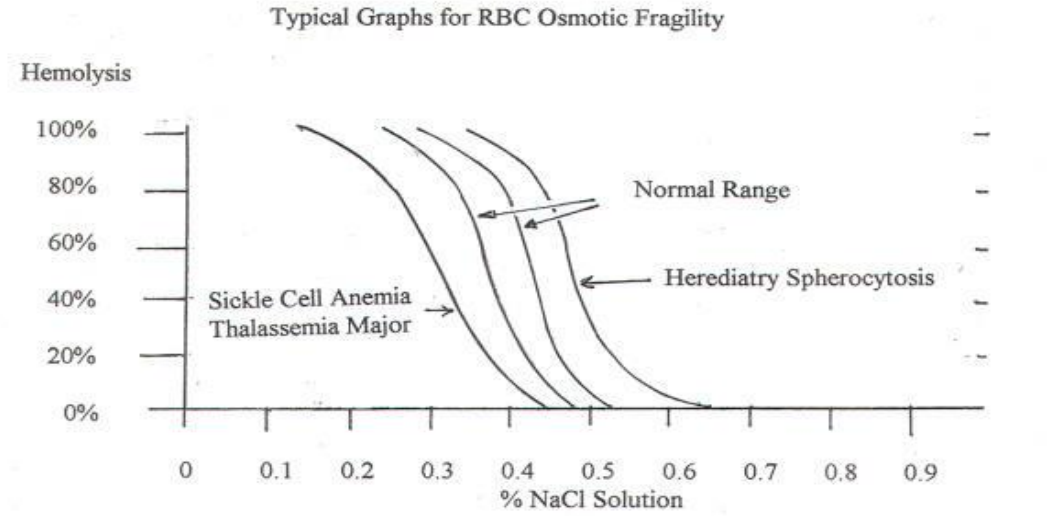
Result: Microcytosis, hypochromia and anisopoikilocytosis (variation in the size and shape of the red cells) are the most typical changes in thalassaemia. A high percentage of target cells are found in HbC syndromes. Nucleated red blood cells are indicative of bone marrow hyperactivity and can be found in homozygous β-thalassaemia.

4.4.2.3. Single tube osmotic fragility test (OFT)/ NESTROFT

Single tube osmotic fragility test (OFT)/ NESTROFT:

Osmotic fragility test (OFT) was the first method used for screening of thalassaemia and was introduced as a simple approach to detect thalassaemia carriers by Silvestroni and Bianco in the1940s. The availability of electronic counters for the measurement of MCV and MCH has decreased the use of OFT. It is still used in low resource countries to screen large rural or tribal populations.

The most used test at present is NESTROFT,the acronym for Naked Eye Single Tube Redcell Osmotic Fragility Test [167,168,169]As its name implies, NESTROFT is used to assess osmotic fragility of red cells at a single concentration of buffered saline (0.36% in single tube) visually without a spectrophotometer.



Definition:

Osmotic fragility is a test to measures red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions. The sooner hemolysis occurs, the greater the osmotic fragility of the cells[151] . The classic osmotic fragility test, originally described by Parpart et al (1947)[152].The osmotic fragility test (OFT) is used to measure erythrocyte resistance to hemolysis while being exposed to varying levels of dilution of a saline solution.

Use :useful screening tool in the diagnosis of beta thalassemia trait., congenital hemolytic anemia and hereditary spherocytosis.

Time duration/Incubation of erythrocytes: at 37°C for 24 hours increases the test's sensitivity
Specimen: Whole blood (6 mL [2 mL minimum])

Container / Collection Medium: Lavender top (K2 EDTA) tube .(Na Heparin tube or Lithium Heparin tube)

Method: Routine venipuncture
Handling instructions: Specimen must be fresh. Deliver to laboratory after collection and processing within 72 hours, preferably within 24 hours.

Methodology: Spectrophotometer

Material/Reagent : 0.36% buffered saline (BS) prepared by diluting 36ml of 1% buffered saline with 64ml of distilled water (DW) to make 100 ml (Test Reagent).
Principle of NESTROFT:

Normally, red cells put in saline solution begin to lyse at a saline concentration of 0.4-0.5% and lysis is complete at 0.32%. However, in beta thalssemia trait, due to alteration in osmotic resistance of the affected RBC's due to volume/surface area ratio changes, [4] lysis begins at a saline concentration between 0.4-0.35% and it may not be completed even at 0.1% solution. NESTROFT is done at a saline concentration of 0.36%.

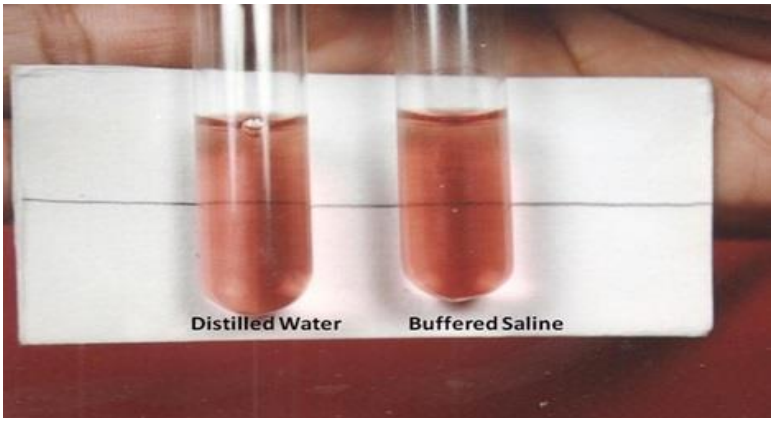
Procedure of the test:

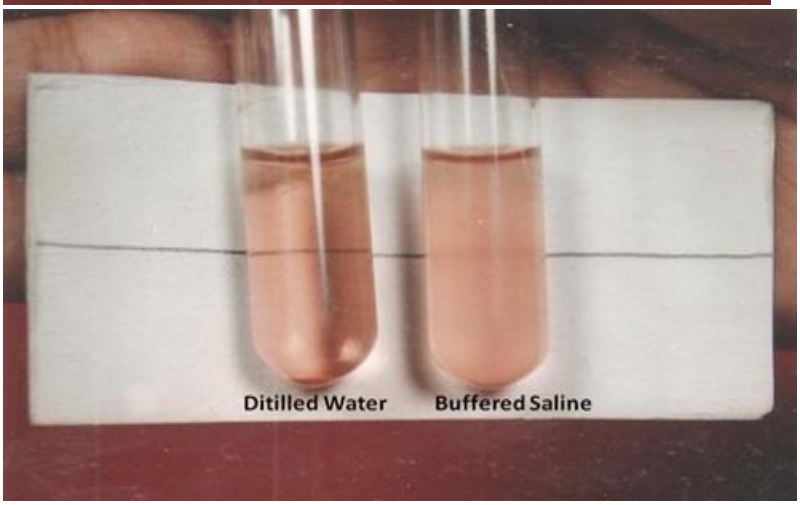
Two test tubes labelled as BS (2ml) and DW (2ml) were taken and a drop of blood was added to each of the tubes, which were then left undisturbed for half an hour at room temperature. Following this, contents of both tubes were gently shaken and held against a white paper on which a thin black line was drawn. The line was clearly visible through DW tube and if it was the same in BS tube; it was considered negative, otherwise test result was interpreted as positive.(Fig)

Results:

Normal Results: A negative test is normal

Photograph showing negative NESTROFT





Photograph showing positive NESTROFT

Advantage:

1. Inexpensive test
2. Some studies[153] showed that NESTROFT is a valuable, cost-effective screening test for beta thalassemia trait and appears to be a valid test in rural setting with financial constraints
3. Requires a small amount of blood
4. Does not require sophisticated equipment
5. Can be applied on the population as a whole is preferred.
6. Many authors in India have found NESTROFT as a suitable test to identify carriers of beta thalassemia trait, especially in rural settings[154]

Disadvantage/Risks:

1. Obtaining a blood sample from some people may be more difficult than from others
2. Excessive bleeding
3. Fainting or feeling light-headed
4. Hematoma (blood accumulating under the skin)
5. Infection (a slight risk any time the skin is broken)
6. The red cell morphology in beta thalassemia trait is microcytic hypochromic; these patients are often misdiagnosed, as those suffering from iron deficiency anaemia and given unnecessary iron medication.

Reference values:

- 0.50 g/dL NaCl (unincubated): 0%-47.8% hemolysis (males), 0%-31.1% hemolysis (females)
- 0.60 g/dL NaCl (incubated): 18.7%-67.4% hemolysis (males), 10.9%-65.5% hemolysis (females)
- 0.65 g/dL NaCl (incubated): 4.4%-36.6% hemolysis (males), 0.2%-39.3% hemolysis (females)
- 0.75 g/dL NaCl (incubated): 0.8%-9.1% hemolysis (males), 0%-10.9% hemolysis (females)

Cost: BDT 30

Availablelity in Bangladesh: Available

4.4.2.4. Dichlorophenol Iodophenol Precipitation (DCIP) Test

The detection of haemoglobin E is important for the antenatal diagnosis of disorders of globin chain synthesis because its interaction with β thalassaemia produces a compound heterozygous state that varies in severity from thalassaemia minor to, more often, thalassaemia intermedia or thalassaemia major.

DCIP: **Dichlorophenol Iodophenol Precipitation Test**

Definition : A simple visual test for the detection of haemoglobin E has been proposed, based on a serendipitous observation when a blue dye, 2,6-dichlorophenolindophenol (DCIP) was used in testing for red cell enzyme deficiencies[158,159] .It was noted that solutions containing haemoglobin E (from patients with either haemoglobin E heterozygosity or homozygosity) became turbid when exposed to this dye.

Use: HbE detection

Reagents: DCIP Reagent:

Tris base	**4.36 g**
EDTA $Na_2.2H_2O$	**2.68 g**
DCIP (Sigma)	**0.0276 g**
Saponin	**0.05 g**

Procedure: (Dissolve in distilled water and adjust pH to 7.5 by 6 M HCl and adjust the volume to 500 ml. This working solution should be kept in 4^{o}C.)

1. Mix 20 ul packed red cell with 5 ml DCIP reagent in 13x100 test tube
2. Incubate the mixture at 37C water bath for 60 min.
3. Look for precipitation before or after addition of 5% ascorbic acid

Method: [155]

1. Add 30 µl of whole blood or 20 µl packed red cell into 5 ml DCIP solution. Gently mix and incubate at 37°C for 1 hour
2. Dissolve in distilled water and adjust pH to 7.5 by 6 M HCl and adjust the volume to 500 ml. This working solution should be kept in 4°C.

Interpretation:

Precipitated hemoglobin can be visualized by the naked eye at the bottom of the tube

Results and comments:

1. **Negative : No precipitation**

2. **Positive : Precipitation seen**

3.	**Normal**	**Negative**
4.	**Homo E**	3^{+}-4^{+}
5.	**HbE trait**	1^{+}-2^{+}
6.	**b-thal/HbE disease**	1^{+}-2^{+}
7.	**HbH disease**	1^{+}-2

In homozygous Hb E, a heavy sediment will form at the bottom of the test tube. In Hb E trait, and Hb E/β-thalassaemia, the precipitation of Hb E produces a cloudy or an evenly distributed particulate appearance. The test is positive also in Hb H disease.

Advantages:

In countries with limited health resources, the use of this test would reduce the number of samples requiring referral to a central laboratory for definitive tests.

Disadvantages:

1. DCIP test is more difficult to read than a sickle solubility test.
2. It is not recommend the use of this screening test in countries where HPLC or haemoglobin electrophoresis on all relevant samples is affordable.

Availablelity in Bangladesh: Not Available

Available in other countries: This test has been used in Thailand for antenatal screening in under-resourced areas[156,157].

4.4.2.5. High Performance Liquid Chromatography (HPLC) Test:

In the screening for classical beta-thalassaemia trait, the hallmark is the presence of an elevated level of HbA_2[160.] This necessitates the accurate estimation of Hb A_2 ($\alpha_2\delta_2$). A number of techniques are available for the measurement of Hb A_2. These techniques include haemoglobin separation on cellulose acetate electrophoresis pH 8.9, microcolumn chromatography followed by analysis of elution with spectrometry and high performance liquid chromatography (HPLC)[161,162.]

HPLC: High Performance Liquid Chromatography (formerly referred to as high-pressure liquid chromatography)

Reagent:
Sample: Venous Blood in any Anticoagulant.
Principal: In this method phosphate buffers at different concentrations (mobile phase), pass under pressure through an ionic exchange column (stationary phase) and the haemoglobins are separated according to their ionic interaction with the stationary phase.
The separated haemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance (415 nm) are measured;

Use:
1. Haemoglobin pattern analysis
2.The most used methods for HbA_2 measurement are CE, HPLC.
3. High performance liquid chromatography (HPLC) has emerged as the method of choice for quantification of HbA_2, HbF and for detection and quantitation of the Hb variants, particularly those which may interact with β-thalassaemia such as HbS, E, C, O-Arab, D and Lepore.
4. For the screening of beta-thalassaemia, the measurement of Hb A_2 should be done by automated HPLC where a level of Hb A_2> 4.0 would be indicative of classical beta-thalassaemia trait.

Advantage:
1. These methods are very accurate, fast and simple
2. HPLC identifies and measure many variant haemoglobins, including the commonly encountered variants HbS, HbC, HbE and Hb D-Punjab
3. In one equipment in one shift of 8 hours 300 test can be done,

Disadvantage:
1. High Cost of equipment and reagent (cost of Equipment BDT and reagent per test cost BDT

Cost: BDT 700/= per test
Availablelity in Bangladesh: Available
4.4.2.6. Electrophoretic Method
Electrophoresis is a separation technique based on the mobility of ions in an electric field. It is the classical method of identifying and quantifying the haemoglobin proteins.

The haemoglobin molecules (HbA, HbA_2, HbF and variants) in solution are electrically charged at any given pH. They can have a positive charge or a negative one according to the ion stable groups (acidic or basic side chain) that they have. Total haemoglobin, which is a mixture of these molecules, has a net negative charge. When an electrical potential difference is applied, particles will migrate either to the cathode or the anode depending on their net charge, and molecules with different overall charges will begin to separate.

Classification of methods: There are several electrophoretic methods, mostly classified according to the supportive media.
The supportive media are described as:
1. Liquid - the only “free” method still used to separate haemoglobin molecules is capillary electrophoresis.
2. Solid - this includes paper, which is no longer used and cellulose acetate, which is one of the most commonly used media.
3. Gel - such as starch (which is also no longer in use), agar, agarose, and polyacrylamide.

Capillary Electrophoresis for Haemoglobinopathies Screening

Capillary Electrophoresis is an emerging diagnostic tool in many clinical chemistry labs to separate Hb fractions and calculate the percentage of each fraction. A commercially available apparatus, used in several European labs, is for example the Capillarys from Sebia (Lisses, France).

Sample: The anticoagulant in EDTA, but the use of citrate or heparin is also acceptable.

Use: The Capillarys recognizes automatically the A_0, F, C and A_2 peaks. Other peaks have a specific position (zones) for instance HbS, HbD, HbE, HbH and HbJ.

Advantage:

1 These methods are very accurate, fast and simple
2 CE identifies and measure many variant haemoglobins, including the commonly encountered variants HbS, HbC, HbE and Hb D-Punjab
3 In one equipment daily in 8 hour shift 200 tests can be done by single technician.

Disadvantage:

1 High Cost of equipment and reagent (cost of equipment BDT and reagent per test cost BDT)

Cost: BDT 1250/= per test

Availablelity in Bangladesh: Available

Cellulose Acetate Electrophorosis

This is an acetate salt of cellulose produced by treating cotton with acetic acid using sulphuric acid as a catalyst.

Use : Cellulose acetate electrophoresis may be used for qualitative identification of variants, but also with elution for quantitation of the haemoglobins, A_2, A, S, D, Lepore, α-chain variants, HbH and Hb Bart's.

Reagents and materials:

1. Tris-EDTA Boric Acid (TEB) buffer, pH 8.4
2. Whatman No. 3 chromatography paper.
3. Cellulose acetate membranes

4.4.3 Prenatal Diagnosis

Amniocentesis and chorionic villus sampling for Prenatal Thalassemia Testing allows identification of Thalassemia in unborn children.

4.4.3.1 Chorionic Villus Sampling

Definition: Chorionic Villus Sampling(CVS) is a diagnostic test carried out during pregnancy, which involves removing tissue from the placenta.

Indication:

1. CVS is used to detect chromosomal abnormalities such as Down's syndrome.
2. It can also detect certain other genetic conditions, such as sickle cell disease and thalassaemia major.

Time for testing Chorionic villus sampling

It is therefore recommended that CVS be carried out after the beginning of the 9th week of gestation at the earliest, and preferably from 101/2 weeks' gestation.

Procedure for CVS

A small amount of local anaesthetic is usually injected into skin. During transabdominal CVS, an ultrasound scan is used to check the position of the baby. A fine needle is then inserted through abdomen and into womb. A tiny sample of tissue is then removed from placenta (placenta contains tissue that is genetically identical to baby). The needle does not enter the sac (or bag of fluid) around the baby. After CVS, the hospital sends the tissue sample from placenta to a laboratory for testing. The tissue sample contains some the baby's cells. Cells contain a person's genetic information. Two types of laboratory test are used to look at baby's chromosomes – 'PCR' (polymerase chain reaction) or a 'full karyotype'.

Time: The test itself takes around 10 minutes.

Advantage:

1. CVS has great advantages because it can be done in the first trimester of pregnancy,
2. CVS gives most reliable results during the first trimester of pregnancy
3. It is the preferred sampling method for DNA analysis of the haemoglobinopathies.

Disadvantage:

1. Most women say that having CVS is uncomfortable rather than painful.
2. Vaginal spotting or bleeding is the most common immediate complication following a CVS procedure (1-4% of cases) and is often observed after a transcervical sampling (in up to 20% of cases) [165]
3. Direct vascular injury of small branches of the utero-placental or umbilico-placental circulation may also lead to a retro-placental haematoma and/or a subchorionic haemorrhage and subsequently to a miscarriage.(1 to 2%)
4. Intrauterine infection and chronic amniotic fluid leakage are two other possible complications, occurring a few days to 3 weeks after the procedure.

***Precaution Needed During CVS*:**

1. If some ones blood group is rhesus negative, she will be offered an anti-D injection after this procedure.
2. Because CVS is an invasive test, the hospital will want to know whether or not she has HIV or hepatitis B

Cost: BDT 18,000/=

Available in Bangladesh: Dhaka Shishu Hospital Thalassaemia Center has recently started a DNA lab analysis of chorionic villus and amniotic fluid sample to detect the status of fetus where the parents are carriers of the thalassaemia[166]

4.4.5.2 Amniotic fluid sampling: (**Amniocentesis)**

Mid-second trimester (15-19 weeks) amniocentesis was introduced in the 1960s and is still the most commonly used invasive technique for the prenatal diagnosis of genetic defects of the foetus.

Indication: prenatal diagnosis for detection of thalassemia.

Time for amniotic fluid sampling: Amniocentesis is conventionally done from 16 weeks' gestation onwards.

Procedure: The procedure involves withdrawing 15-20ml of amniotic fluid from the amniotic cavity surrounding the foetus, using a small needle inserted through the abdomen under continuous ultrasound guidance. After collection of sample, DNA analysis is done to determine weather the fetus is affected, carrier or normal. If the fetus is affected which means that s/he will be born with thalassaemia the choice is with the parents to abort or carry on with the pregnancy. [166]

Advantage: Early detection of thalassemia carrier.

Disadvantage:

1. The main disadvantage in this method is the lateness of the procedure and diagnosis. Abortion here is psychologically traumatic to the mother. [166]
2. First trimester amniocentesis is considered to carry a higher risk of miscarriage than mid-trimester amniocentesis.

Cost: BDT 6000/=

Available in Bangladesh: Dhaka Shishu Hospital Thalassaemia Center has recently started a DNA lab analysis of chorionic villus and amniotic fluid sample to detect the status of fetus where the parents are carriers of the thalassaemia[166]

4.4.5.3 Foetal Blood Sampling: (FBS)

A needle is passed through the abdominal wall under continuous ultrasound monitoring and inserted into the placental insertion of the umbilical cord, and a small sample of foetal blood withdrawn. This procedure can be safely carried out after 20 weeks of pregnancy. Foetal blood sampling is associated with late diagnosis and requires a high level of expertise. Foetal blood sampling is now rarely used to obtain a sample of foetal blood at a relatively late stage of pregnancy for rapid DNA analysis or globin chain synthesis.

References to all literature reviewed related with Prevalence and Patient Charactieristic and Tools are given at Appendix 7.

CHAPTER-5

PREVALENCE OF THALASSAEMIA/HAEMONOGLOBINOPATHY TRAITS IN BANGLADESH

5.1 Occurrence of Thalassaemia Traits as Identified under This Assignment

In connection with the assignment primary as well as secondary sources were explored for data. As no survey was specified in the ToR a survey on pilot basis was carried on 537 households. Among these households 395 gave blood samples and all others gave information on awareness. In addition they gave opinions civil society and government role which has specifically addressed in Chapter 7. Initially secondary data were collected and put in database and analysed. Haemoglobin (Hb) was tested against the characteristics related with carriers and diseases. The classification made is given here under:

Table 5.1: Haemoglobin Characteristics of Blood Samples.

Sl. No.	Haemoglobin Count by Type	Percentage of types of Haemoglobin	Result diagnosed	Sl. No.	Haemoglobin Count by Type	Percentage of types of Haemoglobin	Result Diagnosed
1	Hb A	96.8.- 97.8	Normal	5	Hb A	<96.8	E β Thalassaemia disease
	Hb A2	2.2.- 3.2			Hb A2	variable	
2	Hb A	<96.8	Hb E Carrier		HbE	>11	
	Hb A2	Variable			Hb F	>1.0	
	Hb E	>11.0		6	HbA	<96.8	β Thalassaemia disease
3	Hb A	<96.8	β carrier		HbA2	variable	
	Hb A2	>3.2			HbF	HbA	
4	Hb A	<96.8	Hb E Disease				
	Hb A2	Variable					
	Hb E	Hb E> Hb A					

The graph created by electrophoresis machine depicting the values of haemoglobin by type is shown graphically. **Figure 5.1** shows sample graph for a Normal Blood as classified above. Examples of other graphs are given at 6. The test results are depicted in the following sections.

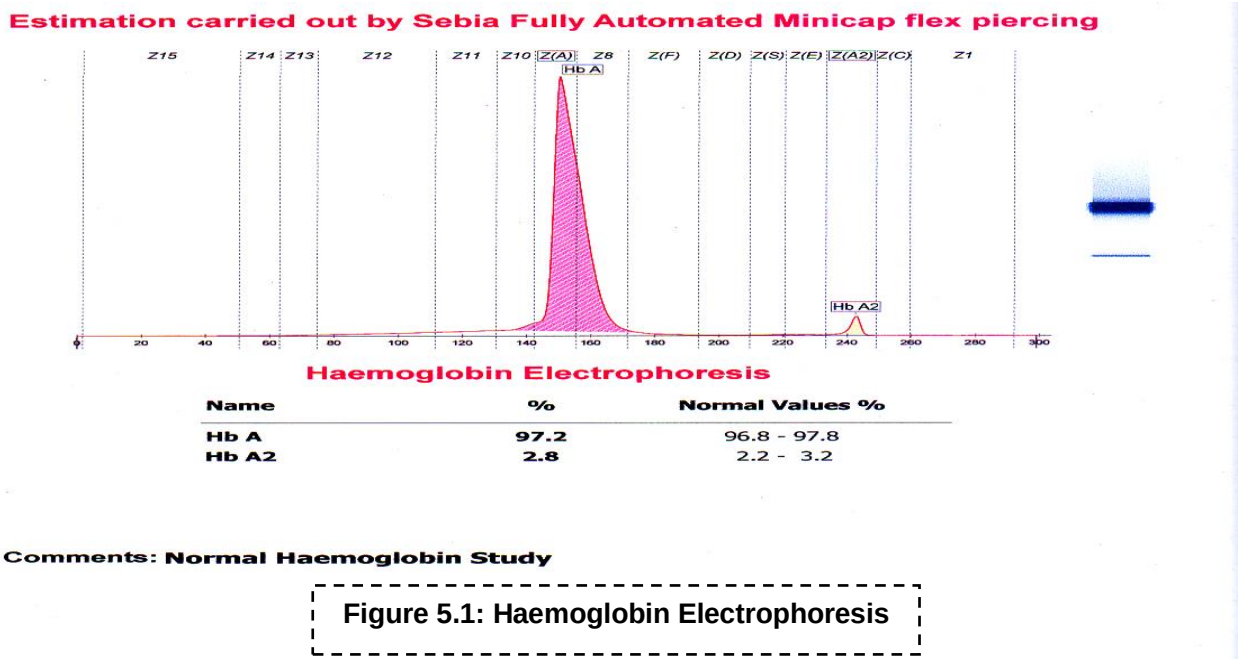


Figure 5.1: Haemoglobin Electrophoresis

A total of 395 individuals were included in the study. The samples were collected from colleges and universities located in Dhaka city during April- May 2016**.** The samples were analaysed at Bangladesh Thalassaemia Samity Hospital Laboratory.

About 2-3 ml intravenous blood samples were collected after obtaining informed consent using EDTA (ethylene diamine tetra acetic acid) tube as anticoagulants by disposable syringes and needles from each individual.

The Sysmex XI-800 system Haematology analyzer (Sysmex Corporation, Kobe, Japan) was used to determine peripheral cell count and red blood cell indices (RBC, Hb%, HCT, MCV, MCH, and MCHC) using standard procedure [4] that employed RF/DC detection method, hydrodynamic focusing, flow cytometry method and SLS-haemoglobin method.

Haemoglobin electrophoresis was carried out on capillaries electrophoresis using the K20 System (Sebia, Issy-les-Moulineaux, France).

5.2 Occurrence as found from Data obtained from Secondary Sources

5.2.1 Occurrence of Thalassaemia Traits in Bangladesh: Blood samples collected from volunteers were tested by surveys undertaken previously under different programmes in Bangladesh in a very limited scale. Secondary data were mainly collected from the database of BTS. In addition data from Shishu Hospital were collected.

The occurrence of Thalassaemia had of late been attracting the notice of different organizations. The attention received by this non contagious disease was much less than other similar sort of diseases. Some of those diseases are not preventable or hard to prevent; on the other hand this disease is preventable by simple management initiative. Some organizations took note of the situation and came forward to understand the situation in a sporadic manner.

These institutions use electrophoresis instrument for testing blood samples. Though there are other methods of testing but reagents for such testing are not available. Only NESTROFT a synonym of Thalachip is available. But this is suitable for detection β Thalassaemia trait only. DCIP used for Hb E Thalassaemia is not available in the country. Thalassaemia International Foundation (TIF)

recommends electrophoresis as the only method that may give proper test results of all types of Hemoglobinopathy faced in Bangladesh.

Blood test was carried out among student volunteers mainly in Dhaka and once in Noakhali and once in Bogra. The districts of origin of all the volunteers were recorded while taking blood samples. The test results of 1439 samples were put in a database, these were analysed and following are the findings.

5.2.2 The Division Wise Distribution of Persons with Thalassaemia Traits

The number of volunteers tested division wise is given in **Table 5.2** below. The number of volunteers is maximal in Dhaka, Rajshahi and Chittagong as samples were collected in these divisions only. The test results did not only identify trait carrier but small number of Hb E diseased persons. All critical and major complications among patients take place in the childhood. Hb E is not a major problem but may be in danger at some situations like delivery etc.

Table 5.2 Division wise Distribution of Thalassaemia Carriers

Name of Division	Number of Persons Tested to be Normal or Bearers				
	Number tested	Non Thalassaemia	Hb E Trait	β Thalassaemia traits	Haemoglobinopathy Diseased
Barisal	77	69	4	2	2
Chittagong	260	227	21	9	1
Dhaka	475	420	39	12	4
Khulna	159	149	8	2	0
Rajshahi	338	280	51	8	4
Rangpur	116	73	31	5	7
Sylhet	14	12	1	1	0
Total	1439	1230	155	39	18

Tests carried out on persons aged between 12 – 40 years; however most of persons were within the age 20- 27 years. The test depicted the following features. In the country about 14.0% healthy looking young people are carriers of Thalassaemia trait and 1.3% of persons are Hb-E diseased. Figure 5.2 beside shows that the prevalence of Hb-e is as high as 10.8 %. The occurrence of β Thalassaemia is 2.7%. The situation is not uniform through the country. The variation inside each division is described in the following sections.

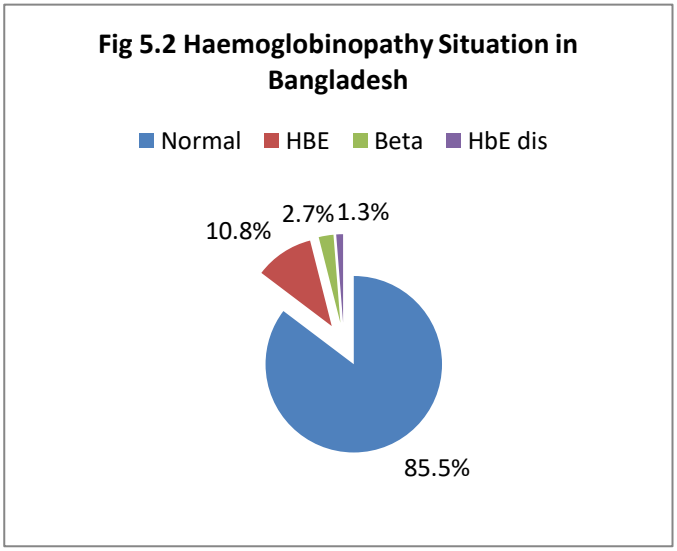


Fig 5.2 Haemoglobinopathy Situation in Bangladesh

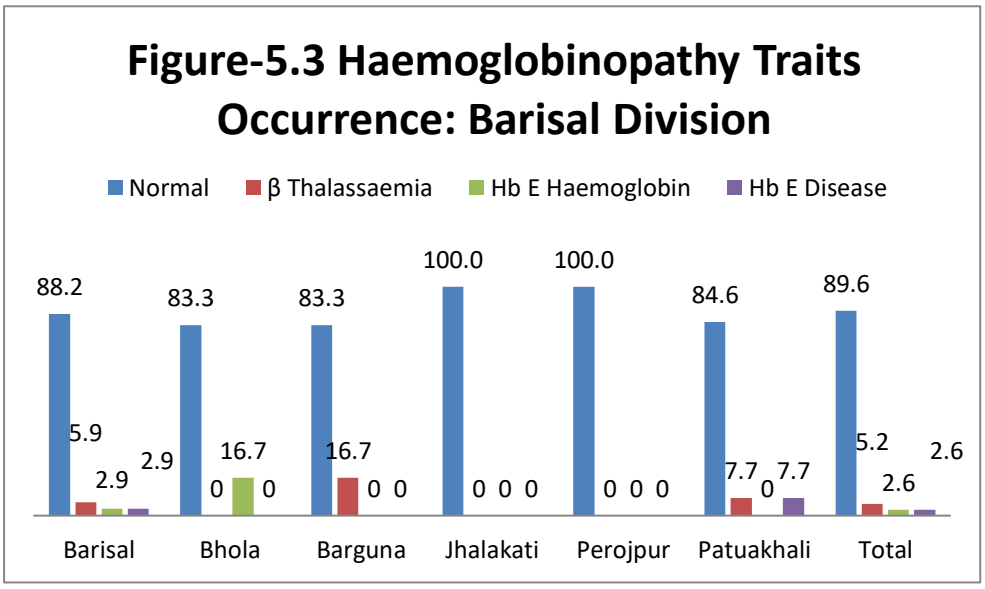
5.2.3 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Barisal Division

In all 77 blood samples could be collected from persons having origin Barisal Division. Among the tested samples 34 were normal while 3 were carriers of β Thalassaemia trait and 2 were carriers of Hb E Trait. The samples included 2 persons having disease (HbE) with intermediate complication. The number of samples from Bhola, Barguna and Jhalakati was only six (6) for each. The results of these districts are to be used with caution.

Table 5.3 District wise Distribution of Thalassaemia Tested Persons in Barisal Division

Hb Test Results	No of Persons tested						
	Barisal	Bhola	Barguna	Jhalakati	Perojpur	Patuakhali	Total
Normal	30	5	5	6	12	11	69
Hb E	2	0	1	0	0	1	4
β Thalassaemia	1	1	0	0	0	0	2
Haemoglobinopathy Disease	1	0	0	0	0	1	2
Total	34	6	6	6	12	12	77

In Barisal Division in all 10.4% persons were either carriers of Thalassaemia or Hb –E diseased. Barisal District had the highest 11.8 % carriers. Among the carriers 5.9% were carriers of Hb E while 2.9% were carriers of B Thalassaemia and 2.9% were Hb E diseased. The situation is depicted in Figure 5.3



5.2.4 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Chittagong Division

In all 260 blood samples could be collected from persons having origin in Chittagong Division. Among the tested samples 227 were normal while 21 were carriers of Hb E Trait and 9 were carriers of B Thalassaemia trait. There was one Hb E diseased person in the sample volunteers. There was no sample from Rangamati District and number of samples in Cox's bazar, Khagrachari and Bandarban ranged between 2 -5 only. These small samples may not be representative of the district. The number of samples is most in Noakhali and adjacent area as blood samples were collected at Noakhali.

Table 5.4: Distribution ofHaemoglobinopathy Traits in Districts of Chittagong Division

TestResults	Chittagong	Cox bazaar	Comilla	Brhaman baria	Chandpur	Noakhali	Feni	Luxmipur	Rangamati	Khagchari	Bandarban	Total
Normal	20	4	58	16	51	48	12	15	0	0	3	227
Hb E	1	1	5	2	4	5	0	0	0	2	1	21
βThalassaemia	0	0	2	1	2	4	0	0	0	0	0	9
Hb E disease	0	0	0	0	0	0	1	0	0	0	0	1
Total	23	5	65	19	57	57	13	15	0	2	4	260

In Chittagong Division, in all 12.07 % persons were carriers of either form of Thalassaemia prevalent in the country. One hundred percent of Khagrachari samples and 25% of Bandarban samples were Hb E carriers respectively. Cox's Bazar had 20 % β Thalassaemia carriers. But the number of samples is small in these districts; so these results are to be considered with caution. Noakhali has about 8.8% Hb E. Carrier and have in addition 7% of beta thalassaemia carriers. Brahman baria has 10.5% of HbE carriers along with 5.3 % of β Thalassaemia carriers. The situation of each district may be visualized from Figure 5.4 above. The sample persons of the division did not have any Hb E diseased persons.

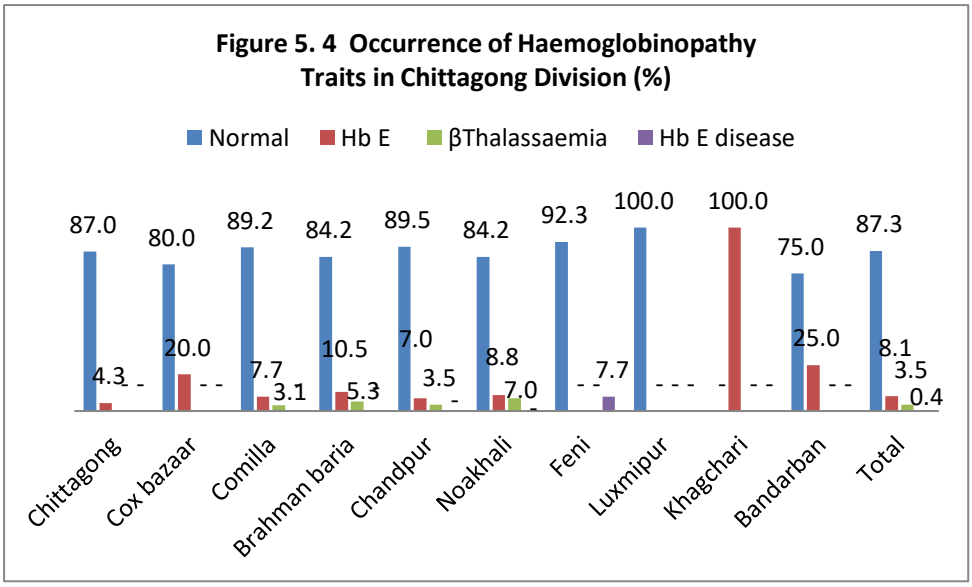


Figure 5. 4 Occurrence of Haemoglobinopathy Traits in Chittagong Division (%)

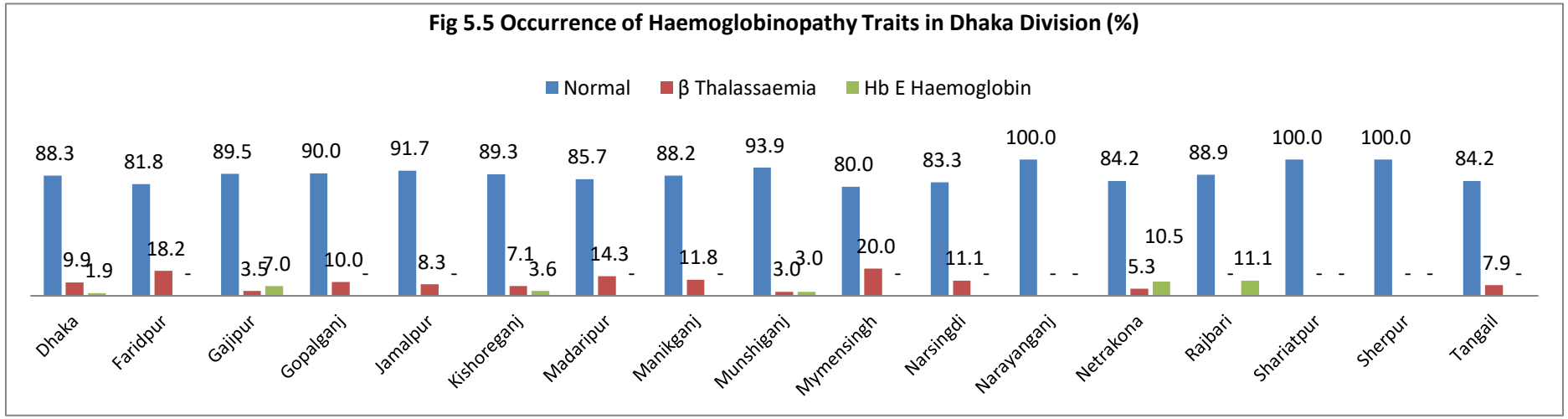
5.2.5 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Dhaka Division

In all 475 blood samples could be collected from volunteers having origin Dhaka Division. Among the tested samples 420 were normal while 39 were carriers of Hb E Trait and 12 were carriers of βThalassaemia trait. There are 4 persons with Hb E disease. The number of samples from Sherpur District was only 3. These small samples may not be representative of the district. The number of samples is most in Dhaka as blood samples were collected in Dhaka. The situation is depicted at Table 5.5 below:

The occurrence of Haemoglobinopathy traits is maximal in Mymensingh District being 20%, followed by Faridpur, Netrokona and Madaripur 18.2%, 15.8% and 14.3% respectively. The situation is depicted at Figure 5.4 above. The occurrence of β Thalassaemia is maximal in Netrokona District while Hb E is maximal in Faridpur District. The Overall Occurrences of Hb E and βThalassademia in the division are 8.2% and 2.5% respectively. The percentage of Hb E Disease is 0.8 % . The Situation is depicted in Figure 5.5 below.

Table 5.5 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Dhaka Division

Type	Dhaka	Faridpur	Gajipur	Gopalganj	Jamalpur	Kishoreganj	Madaripur	Manikganj	Munshiganj	Mymensingh	Narsingdi	Narayanganj	Netrakona	Rajbari	Shariatpur	Sherpur	Tangail	Total
Normal	143	9	51	9	11	25	12	15	31	16	15	14	16	8	10	3	32	420
Hb E	16	2	2	1	1	2	2	2	1	4	2	0	1	0	0	0	3	39
B Thalassaemia	3	0	4	0	0	1	0	0	1	0	0	0	2	1	0	0	0	12
Hb E Disease	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	4
Total	162	11	57	10	12	28	14	17	33	20	18	14	19	9	10	3	38	475



5.2.6 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Khulna Division

In all 159 blood samples could be collected from volunteers having origin Khulna Division. Among the tested samples 149 were normal while 8 were carriers of Hb E Trait and two (2) were carriers of β Thalassaemia trait. The number of samples is most from Jessore District. The situation is depicted at **Table 5.6** below:

Table 5.6 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Khulna Division

Blood Character istic	Bagerhat	Chua danga	Jessore	Jhenai daha	Khulna	Kustia	Magura	Meherpur	Narail	Satkhira	Total
Normal	14	16	29	14	14	19	5	3	10	25	149
Hb E	0	0	4	1	0	1	2	0	0	0	8
BThalassaemia	0	0	0	0	0	0	2	0	0	0	2
Hb E Disease	0	0	0	0	0	0	0	0	0	0	0
Total	14	16	33	15	14	20	9	3	10	25	159

The occurrence of Haemoglobinopathy is maximum in Magura District being 44.4%. Meherpur, Khulna, Chuadanga, Narail and satkhira had no bearers of the traits. But samples Meherpur is only 3; so, the result may not be representative of the district. It is followed by Jessore (12.1%). and Jhenaidaha (6.7%) two adjacent districts. The situation of the division as a whole is depicted in Figure 5.6 shown above.

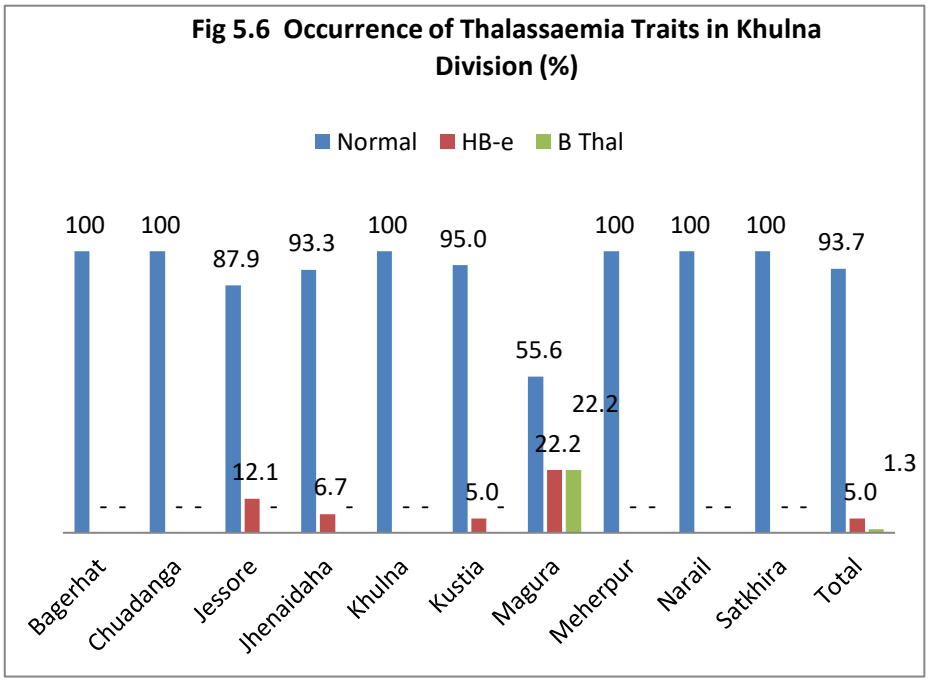


Fig 5.6 Occurrence of Thalassaemia Traits in Khulna Division (%)

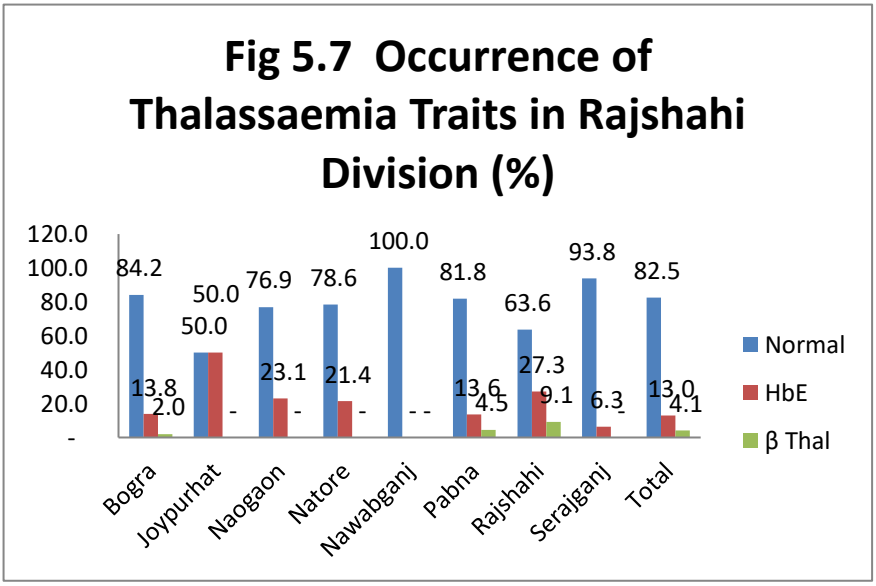
5.2.7 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Rajshahi Division

In all 338 blood samples could be collected from volunteers having origin Rajshahi Division. Among the tested samples 275 were normal while 51 were carriers of Hb E Trait and 8 were carriers of β Thalassaemia trait. There were four (4) Hb-E patients. The maximum number of volunteers belonged to Bogra as sample collection was done once in Bogra. The situation is depicted at **Table 5.7** below:

Table 5.7: Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Rajshahi Division

Test Result	Bogra	Joypurhat	Naogaon	Natore	Nawabganj	Pabna	Rajshahi	Serajganj	Total
Normal	205	1	10	11	2	18	13	15	275
Hb E	34	1	3	3	0	3	6	1	51
BThalassaemia	5	0	0	0	0	1	2	0	8
Hb E Disease	3	0	0	0	0	0	1	0	4
Total	247	2	13	14	2	22	22	16	338

Rajshai Division has 17.1% Haemoglobinopathy trait bearers. The Hb E trait bearers are 13% while remaining 4.1% are β thalassaemia trait bearers. The situation of the division as a whole is depicted in Figure 5.7 below: In Joypurhat the occurrence of trait bearers was high (50%) but the number of samples is only two (2). Similarly in Nawabganj District there is no case of trait bearer. The number of samples here is also only two (2). These results may give some indications but may not be representative of the districts. The percentage of trait bearers in Rajshahi, Natore and Naogaon Districts are 36.4%, 23.1% and 21.3 % respectively.

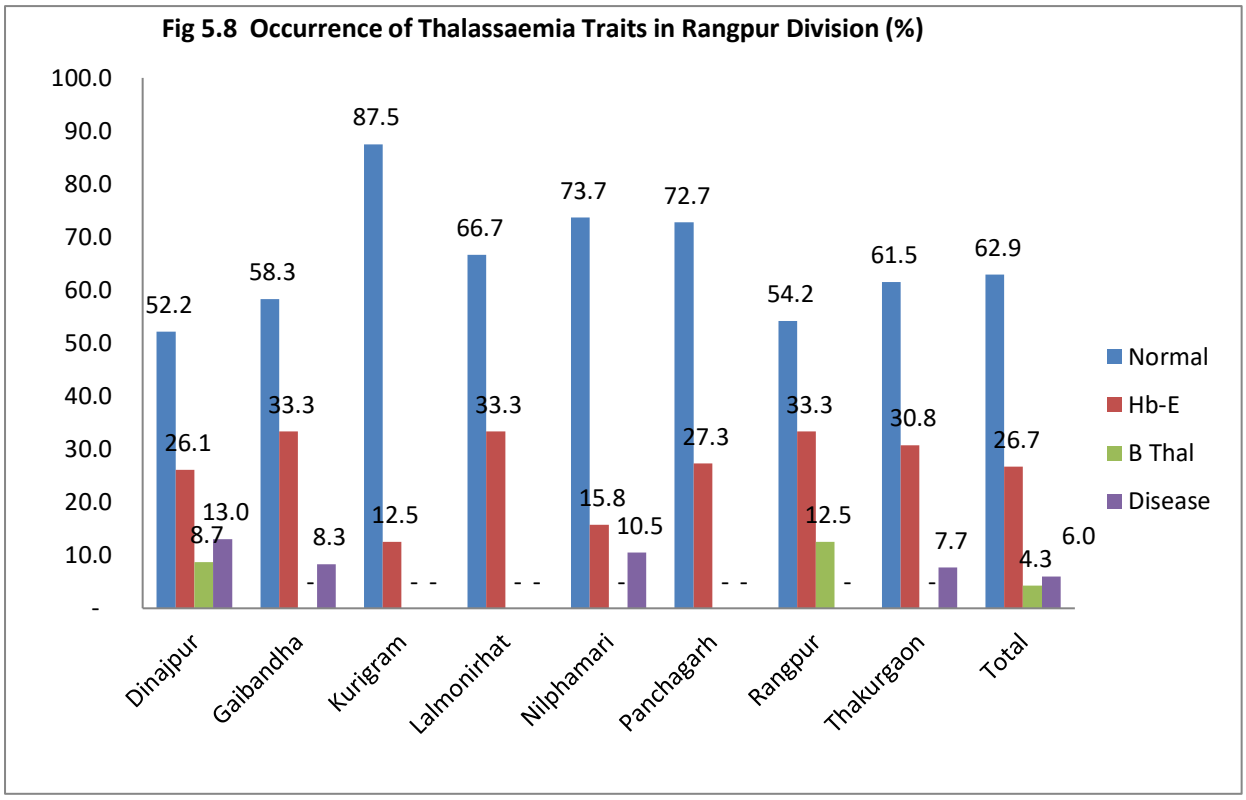


5.2.8 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Rangpur Division

In all 116 blood samples could be collected from volunteers having origin in Rangpur Division. Among the tested samples 116 were normal while 31 were carriers of Hb E Trait and 5 were carriers of β Thalassaemia. There is 7 Hb-E diseased person. The maximum number of volunteers belonged to Rangpur followed by Dinajpur. The number of samples in Lalmonirhat was only 6. The situation is depicted at Table 5.8 below:

Table 5.8 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Rangpur Division

Type	Dinajpur	Gaibandha	Kurigram	Lalmonirhat	Nilphamari	Panchagarh	Rangpur	Thakurgaon	Total
Normal	12	7	7	4	14	8	13	8	73
Hb E	6	4	1	2	3	3	8	4	31
βThalassaemia	2	0	0	0	0	0	3	0	5
Hb E	3	1	0	0	2	0	0	1	7
Total	23	12	8	6	19	11	24	13	116



The prevalence of Haemoglobinopathy traits is very high in Rangpur Division. The Hb E trait bearers are 26.7 %. In addition there are 6% persons with HbE disease. The worst situation is found to occur in Dinajpur District where 47.8 % of the people are bearers of Haemoglobinopathy traits. This is followed by Rangpur District (45.8%).The situation is alarming in all districts except Kurigram where the rate is 12.5% which is close to average of Bangladesh. The situation of the districts of the division as a whole is depicted in Figure 5.8 beside.

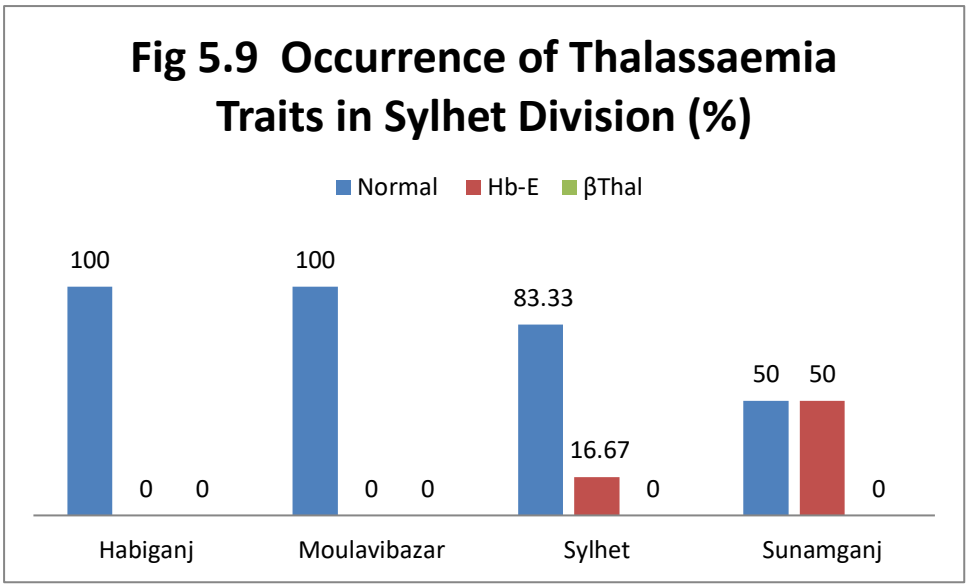
5.2.9 Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Sylhet Division

The blood samples collected contained only 14 persons from four districts of Sylhet Division. Among the tested samples 12 were normal while 1 was carriers of Hb E Trait and 1 was carriers of β Thalassaemia trait. The number of samples in two districts (Moulavibazar and Sunamganj) is only 2 for each and it is difficult to come to any inference from such a small sample. The situation is depicted at Table 5.9 below:

Table 5.9: Distribution of Occurrence of Haemoglobinopathy Traits in Districts of Sylhet Division

Test Result	Habiganj	Moulavibazar	Sylhet	Sunamganj	Total
Normal	5	1	5	1	12
Hb E	0	0	*1*	*0*	1
B Thalassaemia	0	0	0	1	1
Hb E disease	0	0	0	0	0
Total	5	1	6	2	14

NoHaemoglobinopathy trait bearer is found in Moulavibazar and Habiganj districts. Sylhet has 16.67 % Hb E bearer while Sunamganj has 50% βThalassaemia bearer. The situation of the division as a whole is depicted in Figure 5.9. However, the number of samples in Sunamganj is only 2. So this figure may not considered to be representative of the district,



5.3 Male Female Distribution

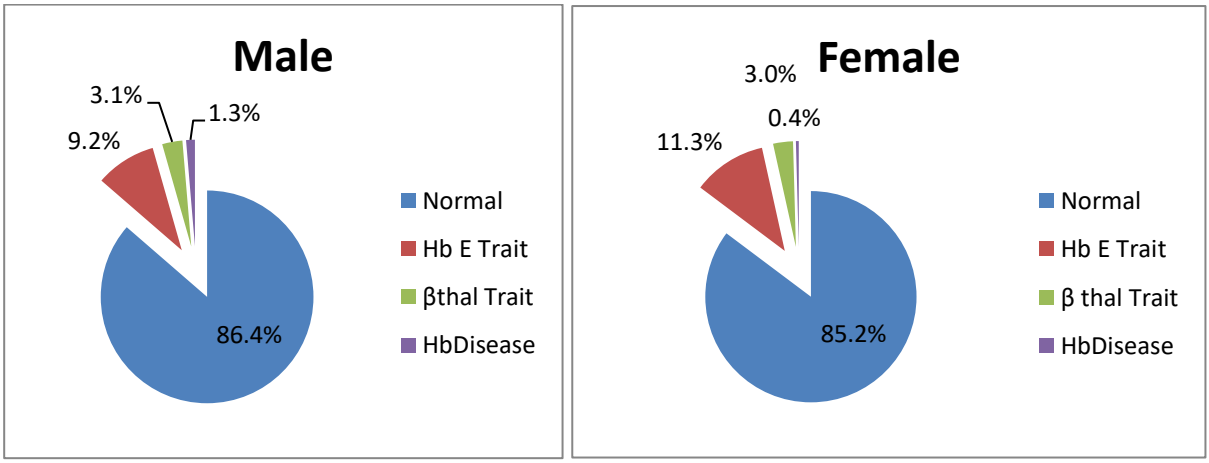
Among volunteers there were male and female volunteers. The number of male volunteers was 748 while the number of female volunteers was 691. The occurrence of Haemoglbinopathy traits among the males and females depict the rate of occurrence is more in males than females but the difference is glaring different. The situation is shown in Table 5.10 below:

Table 5.10 Male/Female Distribution of Haemoglobinopathy Trait Carriers in Bangladesh

Characteristics	Male	Female
Normal	646	589
Hb-e trait	69	78
B Thalassaemia trait	23	21
Haemoglobinopathy Diseased	10	3
Total	748	691

The males had 85.2% normal persons while the females had 86.4 % normal persons. The Hb E trait in male and female was 9.2 % and 11.3% respectively. B Thalassaemia traits were more or less same in males and females, but the Hb E diseased was more (1.3%) in males than (0.4%) in females. The pie chart below depicts the situation in the country. It depicts that there is a sizeable number of Thalassaemia trait carriers in the country and this need immediate attention.

Fig 5.10: Prevalence of Haemoglobinopathy in Males and Females



5.4 Occurrence as obtained from Primary Survey

5.4.1 Occurrence of Thalassaemia Traits as Identified in Survey under the Assignment

In order to have a preliminary idea on the prevalence of Thalassaemia traits in the population of Bangladesh a sample survey was conducted on a pilot basis in Dhaka. Most of the people in Dhaka came in from different parts of the country. While collecting blood samples from volunteers the district of origin of the blood donor was recorded. The test was carried among students of colleges and universities of Dhaka. The volunteers are near future family makers in the country. The results of Thalassaemia test and blood group was given to the volunteers as certificates as an ethical responsibility of the research organization. The institutions wherefrom volunteers were taken are listed in Table 5.11 below:

Table 5.11 Number of volunteers of Educational Institutions who gave blood samples

Name of Institutions	Number of Volunteers	Name of Institutions	Number of Volunteers
1. Dhaka University Arts Faculty	77	2. Dhaka University Curzon Hall	45
3. Jagannath University	55	4. Kabi Nazrul Islam College	25
5. Peoples University, Bangladesh	35	6. Dhaka College	16
7. Bnagladesh University	25	8. Home economic College	15
9. Preparatory College, mohammadpur	15	10. Eden University College	13
11. St. Joseph College	20	12. Mohammad degree College	14
13. Engineering University College	15	14. ASA University	25
15.	242	16.	153

In all 395 blood samples were collected and tested. The test result and basic information of the volunteers were entered in a database. The data collected were analysed to identify the important parameters related with occurrence of trait in the population its regional variation by type of trait the male female distribution.

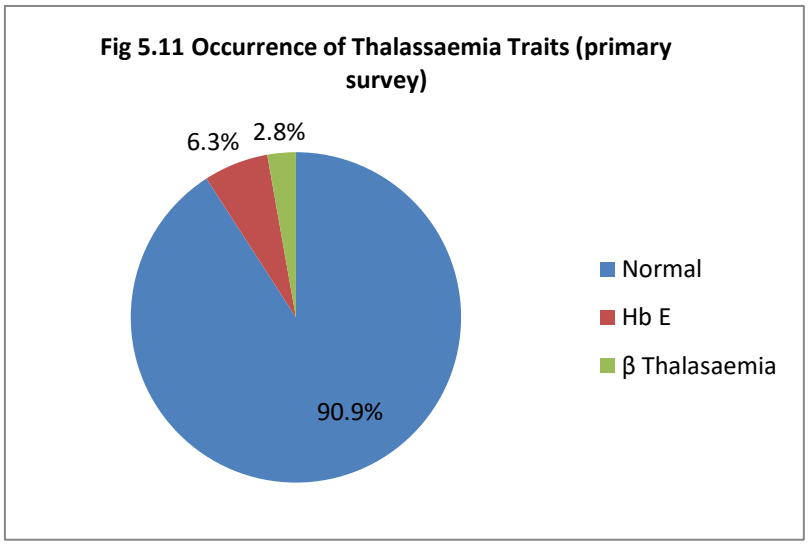
5.4.2 Distribution of Occurrence of Thalassaemia Traits in Different Divisions

The number of volunteers tested division wise is given in **Table 5.12** below. The number of volunteers is maximal in Dhaka as samples were collected in Dhaka only. The test results did not only identify trait carrier but also endeavoured to find Hb E diseased persons. As all critical and major complications among patients take place in the childhood, Hb E does not normally create major problem.

Table 5.12 Division wise Distribution of Thalassaemia Carriers (Primary)

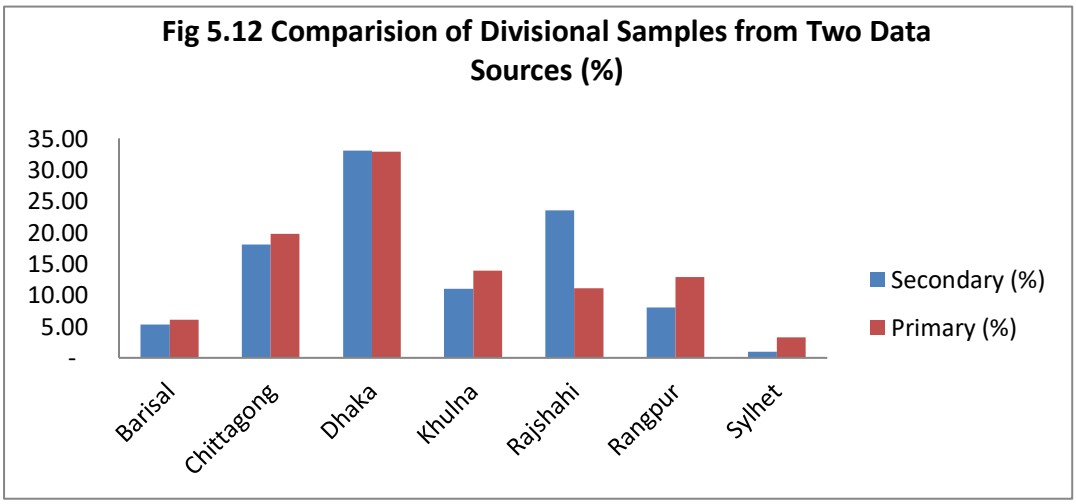
Name of Division	Number of Persons Tested to be Normal or Bearers				
	Number tested	Non Thalassaemia	Hb E Trait	β Thalassaemia traits	Haemoglobinopathy Diseased
Barisal	24	23	1	0	0
Chittagong	78	74	2	2	0
Dhaka	130	120	6	4	0
Khulna	55	51	3	1	0
Rajshahi	44	37	6	1	0
Rangpur	51	41	7	3	0
Sylhet	13	13	0	0	0
Total	395	359	25	11	0

Tests carried out on persons aged between 16 – 38 years, however most of persons were within the age 20- 27 years. The test depicted the following features. In the country a total of 9.1% young people are carriers of Haemoglobinopathy trait. Figure 5.12 beside shows that the prevalence of Hb-e is as high as 6.3 %. The occurrence of β Thalassaemia is 2.8%. The situation is not uniform through the country. The situation is depicted in Figure 5.1.1



5.4.3 Comparison of Data from Two Sources

The prevalence situation in two surveys shows some differences. The prevalence is about 14.5 % from secondary data source while it is about 9.1% from primary data source. This is mainly due to the fact that the survey was undertaken only in a few locations and the samples for each division did not represent the population proportion of the respective division. The secondary data have, almost, tripled the number of data. The occurrence of trait in Rajshahi from secondary sources varies significantly from that primary source data. It is almost similar in other divisions. The secondary data were collected from Noakhali and Bogra in addition to Dhaka. Rajshahi division has, almost 50% less samples in the primary survey. The occurrence of the traits is high in Rajshahi as found in secondary survey. The number of fewer samples has reduced the overall occurrence figure in the country. In addition, incidentally in the primary survey, there was no sample from hill districts, which has high occurrence. These two factors have contributed to over all low rate of occurrence in the primary survey. The percentage of samples from two data sources is depicted in figure below.

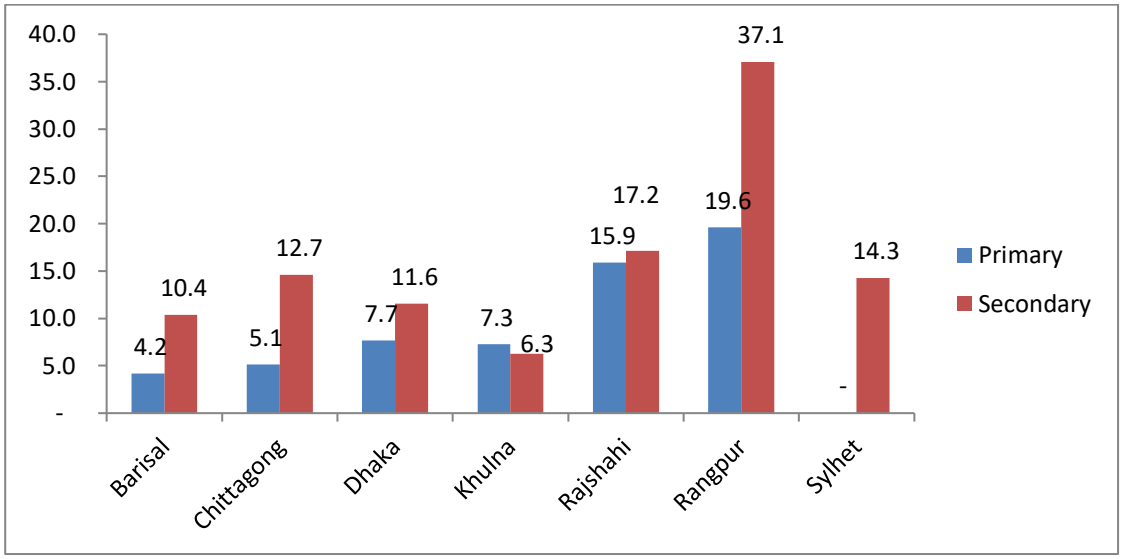


5.4.4 Comparison of Occurrence of Thalassaemia Trait Bearers in two Data Sets

The number percentage of trait bearers in Dhaka, Barisal, Sylhet and Rangpur was much higher in secondary Data source. The reasons include that the secondary data were collected from Bogra and Noakhali for which more local area samples of the respective area was found. The Dhaka residents though from the area might have much social and genetic changes overtime. In the

secondary source data of Chittagong of hill tracts were included and the districts as whole showed high prevalence of the traits whereas there is no sample in primary source from those three districts. Moreover, as the samples were collected on pilot basis without proper sampling there was distortion in the results of two data sets. The percentage of Occurrence in two surveys is depicted in the figure below.

Figure 5.13 Comparison of Percentage of Carriers in Two Data Sets



However, it may concluded with safety that the occurrence of Thalassaemia trait varies in the range between 9-15% of young people of well to do households. The situation is likely to be worse in the country if rural households are included.

5.4.5 Prevalence of Patients

Thesurveys so far carried out by different organizations at different times were mainly on young people rather than children. Thalsaaemia patients do not reach adulthood if not properly treated. Thus the survey could not give any data on patient prevalence in the country. So, an indirect method of estimation of patients was followed based on Hardy –Weinberg equation given below:

From the carrier rates a calculation of the number of affected conceptions per 1000 live births can be made based on the Hardy-Weinberg equation for a recessively inherited single gene disorder. This depends on certain assumptions:

- That the population is mating randomly i.e. there are no consanguineous marriages.
- There is no selection process (e.g. an ongoing prevention program) or genetic drift or frequent spontaneous mutations.

With these assumptions satisfied, the equation is as follows: $p^2 + 2pq + q^2 = 1$

p = thalassaemia gene frequency (½ carrier frequency)

q = Hb A gene frequency = 1-p

p^2 = the frequency homozygotes at birth

pq= the frequency of heterozygotes

q^2 = the frequency for homozygote normals

In case of Bangladesh the birth calculation:

Carrier frequency of 10.2% (Beta Thalassaemia catrrier 4.1 % and HbE carrier 6.1%) from earlier TIF survey.

The gene frequency is approximately 5.6 % or 0.056 (source)

- p = 0.056 and q = 1-0.056=0.944
- The birth rate of homozygotes = p^2
- p^2 = 0.056*0.056 = 0.003136 = 0.3136%
  or 31.36/1000 birth

- 2pq = proportion of carriers born = 2*.056*0.944= 0.105728 ~10.5%
- The proportion of normals at birth = q^2 = 0.944*0.944= .891136 =89.1%
- $p^2+2pq+q^2$ = 0.003136 + 0.105728 + 0.891136 = 1
- Birth Rate = 2.7 %
- Country population = 17,00,00,000 (17 Crore)
- Number of birth = 170000000*0.027 = 45,90,000
- Hence number of births of homozygotes per year = 4590000*31.36/1000= 14394 say 14500

Thus we may conclude that in the country about 14500 children area born every year.

CHAPTER-6

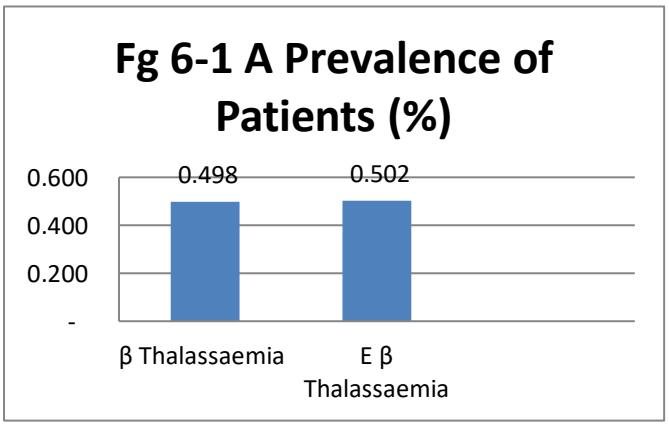
CHARACTERISTICS OF THE PATIENTS

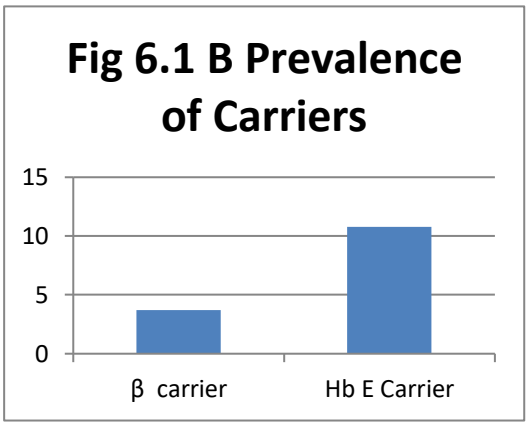
6.1 Prevalence of Patients by Types

In all 215 patients/patients guardians were interviewed and records checked. Only 203 had complete record. From the samples of patients having full record it is found that in the country two types of Thalassaemia occur. These are β Thalassaemia and E- β Thalassaemia. The patients were further subdivided according to the level of the disease characteristics, into major, minor and intermediate. The situation is depicted in Table 6.1. It was found that the occurrence E β Thalassaemia is most in the country resulting to 50.2% followed by β Thalassaemia by 49.8%.

Table 6.1: Occurrence of Types of Thalassaemia

Types of Thalassaemia	No of occurrence (Total Patients = 215)	Percentage
β Thalassaemia	30	14.8%
β Thalassaemia - Major	59	29.1%
β Thalassaemia –Minor	12	5.9%
E β Thalassaemia	52	25.6%
E β Thalassaemia - Major	38	18.7%
E β Thalassaemia- Inter	9	4.4%
E β Thalassaemia- Minor	3	1.5%
Total	**3**	**100.0**

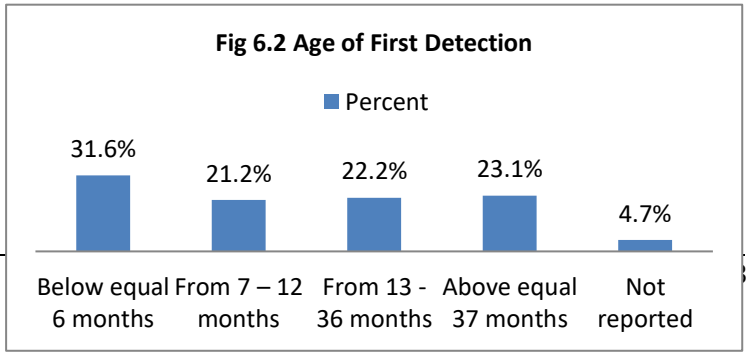




The percentage of patients having β Thalassaemia is almost equal to that of E β Thalassaemia; but the trait bearers of Hb E is almost 3 times more than the that of β Thalassaemia carriers. It indicates that the ratio of β Thalassaemia patients with respect to carriers is more than that of E β Thalassaemia patients with carriers. Alternative it may be said that almost equal number of patients come from both the traits but the number of Hb E trait bearers is almost three times that of the β trait bearers. This is depicted in Figure 6.1A and 6.1 B.

6.2 Age of First Detection

Most of patients (31.6%) were detected of the disease before the age of six months. Next are the patients (23.1%) in the age group more than



36 months. 4.7 % patient households could not tell the age of first detection. The situation is depicted in Figure 6.2.

The most common method was testing by electrophoresis. This method is available in only some selected places mostly in Dhaka and one or two other divisional towns. CBC was used in only 5.1% cases, but it gave indicative values for which almost all had to come for electrophoresis for confirmation. The situation is depicted in Table 6.2

Table 6.2: Distribution of Method Used for Detection of Disease

Method of testing	Percent
CBC	5.1
Electrophoresis	84.7
Both CBC & Electrophoresis	4.2
Others	1.4
No Response	4.6
Total	100

6.3 Symptoms at First Detection

Different symptoms were identified by the guardians of the patients before coming for treatment. Some of them observed more than one symptom. Loss of appetite was report by 61.4% followed by enlarged spleen (51.6%) and improper body growth (48.8%). About 1% of guardians could not specify the symptoms. Jaundice, pale face and Hepatitis, which are almost synonymous was felt by 68.4% of patient's guardians. The distribution of symptoms is given in Table 6.3.

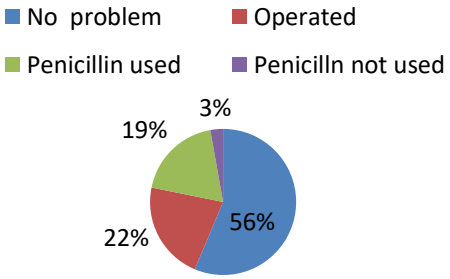
Table 6.3: Symptoms Identified before coming for treatment (multiple response)

Symptoms	No.	%
Improper body growth	104	48.4
Loss of Appetite	132	61.4
Enlarged Spleen	111	51.6
Enlarged Liver	37	17.2
Deep coloured urine	27	12.6
Pale Face	96	44.7
Jaundice	45	20.9
Face Bone deformation	28	13.0
Bone deformation	37	17.2
Hapatitis B	3	1.4
Hapatitis C	3	1.4
No response	2	0.9
Total	**215**	**100**

Most of guardians whose children had enlarged spleen did not consider it to be problematic. Among the guardians of 111 patients only 27.9% (31 patents) considered to be problematic. Really the problem could be understood when operation of spleen was necessary.

Among the patients who underwent spleen operation 87.1% was (19% of total patients with enlarged spleen) undergoing penicillin medication. The situation of spleen problems and medication status is depicted in Fig 6.3.

Fig 6.3 Status of Patients with Spleen Problem



6.4 Treatment and Complications

6.4.1 Blood Transfusion

Blood trasfusion is a sine qua non for Thalassaemia patients for their survival. But it depends on problems of individual patients. So the interval of transfusion is not uniform all patients. The intervals at which blood transfusion are done is indicated in Table 6.4. 61.2% of patients are required to transfuse blood 12 times a year i.e. once each month. 28.5% patients need the transfusion 6 – 12 times a year whereas only 10.3% need transfusion less than six times a year i.e. once in alternate months.

Table 6.4 Frequency of Blood Transfusion

Frequency	No.	%
1-5 Times a Year	22	10.3
6-12 Times a Year	61	28.5
> 12 Times a Year	131	61.2
Total	214	100.0

6.4.2 Type of Blood Transfusion

Whole blood is commonly available but it creates more problems. So, packed and washed cells , are most commonly used. Out of total 215 patients response could be made by 209 patients. The others might have not understood the type of blood used. It is found that 97.6% used packed cell. Among the types washed packed cell, packed cell and packed cell with filter are used by 63.6%, 31.1 and 2.9% patients respectively.

Table 6.5: Type of Blood Used in Transfusion

Type of Blood Transfusion	Number for the Type	% of patients
Whole Blood	5	2.4
Packed cell	65	31.1
Washed Packed cell	133	63.6
Packed cell through filter	6	2.9
Total	**209**	**100.0**

6.4.3 Effects of Iron Overload after Blood Transfusion

Blood transfusion is often cause deposition of iron in different organs of the body for which medicines are required to be taken by the patients to remove excess iron, a process known as chelation. Among the patients having blood transfusion 91.4% informed that medicines are taken by them. The types of medicines taken are depicted in Table 6.6. Most (70.2%) of the patients used Capsule kelfer. No medicine is taken by 3.7% of the patients.

Table 6.6: Medicines used after Blood Transfusion

Type of Medication	No.	%
Injection Despheral	29	15.2
Tab Asunra	12	6.3
Tab Desirox	32	16.8
Cap Kelfer etc.	134	70.2
No Medicine	7	3.7

6.4.4 Side effects of Iron Chelation

Side effects were felt by the patients who underwent medication for chellation of iron. About 72.3 % of patients using medicines gave their opinion in respect of side effects. Among them 10.9% said that no side effect was felt by them. The distribution of those, who faced problems are shown in the pie

Fig 6.4: Problems after Medication

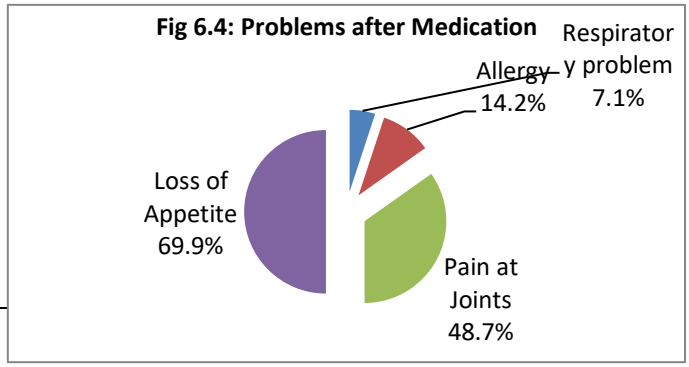


chart given as Figure 6. 4. Loss of appetite is most commonly felt by 69.9% of the patients followed by pain at joint by 48.7%. The other problems faced are allergy (14.2%) and respiratory problems (7.1%).

6.4.5 Problems Faced in the Treatment

Patients faced multiple problems in the treatment. On the whole each patient household identified 1-2 problems. The most common was financial problem stated by 45.1 percent of the households. This figure closely matches with the number of poor households having monthly income less than Tk. 10,000. Non availability of treatment facility is reported by 43.7% of patient household to be second most common problem. Non availability of safe blood is reported by 39.1 % households. Lack of diagnostic facility was expressed by 16.7% households. No response and others accounted for 3.7% households. The situation is depicted at Table 6.7.

Table 6.7 Problems Faced by Patient Households for Treatment

Type of Problems	No.	% of Total
Lack of Treatment Facility in the area	94	43.7
Non availability of safe Blood	84	39.1
Lack of diagnostic facility in the area	36	16.7
Financial insolvency	97	45.1
Others	3	1.4
No response	5	2.3
Total	**210**	**148.4**

6.5 Social Situation

6.5.1 Household Economic Condition

Out of total 215 sample patient households 212 responded to the socio-economic condition of the households. Out of these households 45.3% are from the poor having monthly income of less ran Tk. 10000 per month, while 34.95 are from lower middle class having income in the range of Tk. 10,000 – 25,000 and remaining 19.8 are in the from upper middle class and above. The situation is depicted at Table 6.8.

Table 6.8 Economic condition of Patient Households

Monthly Income level	No.	%
<Tk.10000	96	45.3
Tk 10000- 25000	74	34.9
Tk. 25001- 50000	26	12.3
>Tk 50000	16	7.5
Total	**212**	**100.0**

6.5.2 Opinion of Patient Households on Cost of Treatment

The treatment of Thalassaemia is costly. It requires rigorous follow up and blood transfusion at regular intervals. Out of 215 surveyed patient households 211 households gave response. The cost is found to be expensive by most of the Households. The opinion of patient households on expensiveness of treatment depict that 89.6% of households treat this to be highly expensive while 4.7 % considered that it is not that highly expensive. About 5.7% households considered that it is tolerable. It indicates that poor and middle income households consider this to be expensive. The situation is depicted at Table 6.9.

Table 6. 9 Expensiveness of Treatment

Opinions	No.	Column N %
Highly Expensive	189	89.6
Not so much Expensive	10	4.7
Tolerable	12	5.7
Total	**211**	**100.0**

6.5.3 Social Problems Faced by patients

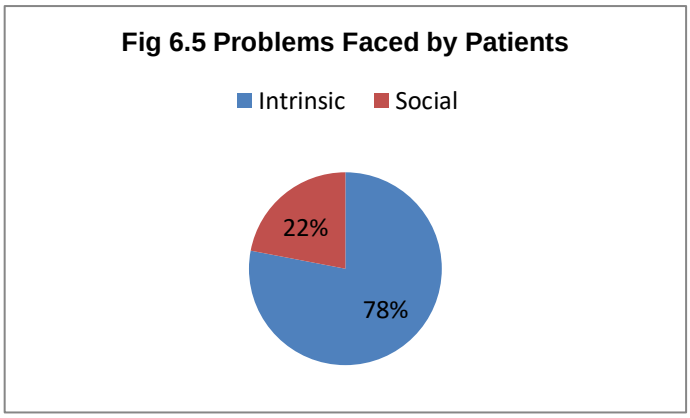
The disease has bad impact on the individual patients. It affects its physique, metabolism and psychology. The patients face various problems some from the people of society while some are intrinsic. Among 215 patients 198 could respond to the query in this study. The problems faced by the patients are shown in Table 6.11.

Table 6.10. Social Problems Faced by Patients

Type of Problems	No.	%	% of N
Individual Non cooperation	30	15.2	14.0
Friendlessness	8	4.0	3.7
Uncertain Future	153	77.3	71.2
Marital uncertainty	41	20.7	19.1
Weakness in education	57	28.8	26.5
Social non cooperation	30	15.2	14.0
Others	1	.5	.5
Total	**198**	**100.0**	**100.0**

Multiple problems were stated by the respondents. On social problems about one and half responses are given by the individuals. Intrinsic problems were more prominent than social problems. The situation is shown at Figure: 6.5.

Fig 6.5 Problems Faced by Patients



CHAPTER-7

AWARENESS ON HAEMONOGLOBINOPATHY/ THALASSAEMIA IN BANGALDESH

7.1 Awareness on Haemoglobinopathy/ Thalassaemia

The level of awareness on the problem is very poor in the country. Not only common people even students of Universities and colleges who are rather highly educated hardly understood the problems. This was also understood through several focus group discussions held at different places of the city. In addition a survey on more than 500 households of college and university going persons depicted that they had hardly any idea of problem. Only persons and staff of NGOs, who are involved with the health management, could give some idea on the problem.

7.2 Awareness among General Households

7.2.1 Knowledge of Thalassaemia as a Disease

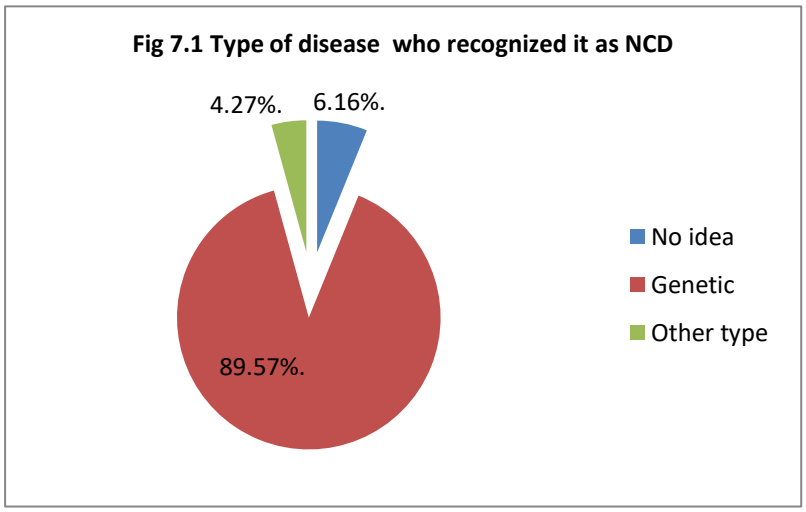
A survey among households with college and university students was carried out with responses from the students. It is found that 51.2% did not know what type of disease it is. 39.3% knew that it was a non-contagious disease (NCD). About 8.9% of the respondents thought it to be contagious and 0.6 % considered it to be psychological disorder.

Table 7.1: Distribution of Knowledge of Thalassaemia

Type of Disease	Response in %
No idea	51.2
Infectious	8.9
NCD	39.3
Psychological	0.6%

The persons who considered it to be psychological disease did not consider it to be genetic or infectious. The situation is depicted at Table 7.1.

Among those, who considered it as NCD 6.16 % could not tell what type of type it was. Among them 4.27 % this considered to be another type of disease. However, 89.6 % of them could tell that it was genetic disease. The situation is depicted at Fig**7.1** Thus among all respondents only 35.2%, 2.4% and 1.7% respectively considered it as genetic, infectious and other type of disease.



7.2.2 Source of Knowledge on Thalassaemia who considered it to be NCD

The source of knowledge of 34.1% household members was family and friends followed by social media (25.1%). The print media accounts for the awareness of about one fifth of the persons. Knowledge from medical professionals is only 2.4%.

Another one fifth had really no Know source, rather it was guess work. The situation is depicted at Table 7. 2

Table 7.2 : Source of Knowledge on Thalassaemia

Source of Knowledge	No	Percent
No idea	19	9.0
Social Media	53	25.1
Print media	40	19.0
Family & friends	72	34.1
Physicians	5	2.4
Others	22	10.4

7.3 Awareness in patient Households

Patient households bear the severe brunt of the treatment of the Thalassaemia patients. It is quite costly. At times the households loss all their capabilities of treatment at some stage and they resign to the fate resulting in the ultimate demise of the patients. This causes an enormous loss to the household and the nation. The expenditure made over the years ends in nothingness of futile efforts. So, efforts are needed to overcome the problem, which is feasible through proper health service support and awareness development.

7.3.1 Awareness in Respect of Type of Disease

The discussion with 215 patient guardians/ Patients reveals that more than 85% of the patient households understood that Thalassaemia is a non contagious genetic disease. They rather have a clear idea about the disease. Another 6.5% understands that it is non contagious but its genetic role is not understood. About 0.5 % of the households still consider it to be contagious disease. About 8% of these households consider this to be physical or other type of problems. The situation is depicted at Table 7. 3.

Table 7.3 Awareness of Patient Households (N=215)

Awareness in respect of contagiousness	No.	%
Contagious	1	.47
Non Contagious (NCD)	14	6.5
NCD but genetic	183	85.1
Physical	13	6.0
No response or Others	4	1.9
Total	**215**	**100.0**

7.3.2 Awareness in respect of birth of Children of Thalassaemia Disease.

In spite of the close interaction with hospital and doctors only 63.7 % of the guardians know that it occurs only when both the parents are Thalassaemia trait bearers. About 7 % consider that occurs like other diseases and have no relevance with the Thalassaemic blood characteristic of the parents. About one third (29.3%) households have no clear idea how the disease occurs. The situation is depicted at Table 7.4.

Table 7.4 Awareness on Role of Thalassaemia Trait Bearing Parents

Probability of having Thalassaemia Child if both parents are trait bearers	No.	%
It is irrelevant	15	7.0
Thalassaemia child may be born	137	63.7
Do not Know	63	29.3
Total	**215**	**100.0**

The patient households were enquired of the symptoms felt at the early stage of disease. Out of the total responding households 94.4% responded to the understanding of the problem. The respondent households mostly gave more than one response. On average about 2 responses were given. The most common response was fever.

7.4 Awareness at the Community Level

In order to assess the understanding of the problem by the community several focus group discussions were held at several places of the city. The date and location of the discussions are given at Table 7. 5.

Table 7.5 Details of FGD

Sl. No	Location	Date	No of participants
1	Biswa Sahitya Kendra, Bangla Motor	9/05 /2016	8
2	Sabuj Unnayan, Elephant Road	26/04/2016	9
3	Hunger Project Office Mohammadpur	7/05/2016	7
4	Dakshin Purba Moneswar Kalyan Samity	30/04/ 2016	10
5	Youth Club of Bangladesh, Lalmatia	20/05/2016	10
6	Asia Pacific University	30 / 05 / 2016	7

The discussions at venues were held with a set of checklist as per Appendix 3 and responses were recorded. The summary of discussions is given here under:

- Every year10 thousand children are born with Thalassaemia. The total Carriers is about 1.5 crore. This is a serious problems and needs proper national plan for preventing the occurrence. A plan and log frame is needed. A vision giving detail plan is needed. The youths may initiate a movement.
- Blood test and medication may help in reducing Birth of Thalassaemia children. Simultaneously it may reduce birth of disabled children caused by blood disorder.
- The civil society may initiate Policy Dialogue. The government may initiate School/institutional based programme. The Qazis (Marriage Registrars) may be included in the motivation. For example the hunger project is committed to support the programme. Support at district or upazila level may be provided by the project.
- A national programme is needed. Blood test and health check up is essential.
- A process which gives reliable and fool proof result should be adopted.
- The capacity improvement of UZ complex is to be included as a part of govt. health policy. The enormity of the problem was not understood by the government previously. Knowledge level of physicians is to be increased
- As Thalassaemia may occur if both husband and wife is Thalassaemia carrier, so the programme for all effective age males or females may serve the purpose initially. In case of marriage if the male or female is a carrier the other should invariably have to undertake blood test.
- The Thalassaemia test results should be given as a separate certificate. This should not be incorporated in the National ID card for the sake of privacy.
- The civil society should come forward with motivational programmes. The government should initiate support to the Thalassaemia patients in all its hospitals.
- Insurance policy may be considered but insurers would not come forward if no profit accrues to them. Monetary condition of patient households would not permit.
- Bangladesh has a very good and well spread medical and health support infrastructure. A suitable plan of utilizing these may help overcoming the problem. It is understood actions taken in some countries have already attained success.

CHAPTER-8

SCREENING TOOLS USED FOR DIAGNOSIS IN THE STUDY

8.1 Tools Used in Testing and Their Merit

Haematological methods contribute the basis of identification of carrier of the thalassaemias. Red cell indices and morphology, HbA2 quantification and Hb fraction separation are all used. These are methods often used as conventional screening methods or as elements in the description of the carrier status.

In order to find methods suitable in the country for determining the trait bearers and diseased persons three available testing tools were used. All tests were ultimately compared with CE electrophoresis instrument, which is recognized by the international Thalassaemia Foundation as the most reliable method so far available.

8.2 Comparison of NESTROFT with Electrophoresis

Accordingly 102 samples were tested simultaneously with NESTROFT method and by electrophoresis. The test result shows that NESTROFT test identified in addition to 91 common samples 9 samples as normal though CE identified those 9 as Hb E carriers. It however identified beta thal carriers properly. The situation is depicted at 8.1

Table 8.1 Comparison of test results by NESTROFP and Electrophorasis

Sl No	Category	Nos identified by CE	NESTROFT	Diff
1	Normal	91	100	-9
2	HBE	9	0	9
3	Beta Thal	2	2	0
		102	102	

It transpires from the results that NESTROFT is not capable of detecting HBE carriers and identifies some normal as Carriers. It could however detect Beta thalassaemia properly**.**

The NESTROFT method relies mostly on human visual observation while CE fully automated. The cost of testingone sample including personnel charge is around Tk 25 per tested individual by NESTROFT. Here the cost of the reagents is negligible and may Tk. 2.0 per sample.

8.3 Comparison of CBC with Electrophoresis

Here 110 samples were tested by two test tools. The first was CE Electrophoresis and second By CBC. Both the tools are fully automated. The comparison of results depicts that in the samples 7 were carriers of one or other type trait.

Table 8.2 Comparison of results by two methods

Sl No	Category	Nos identified by CE	CBC	Number	Difference
1	Normal	103	Normal	103	0
2	HBE	5	Abnormal	7	0
3	Bets Thal	2			0
	Total	110		110	0

The CBC properly identified that 7 of the 110 samples had blood disorders indicated by abnormal. It failed to identify the type of disorder. The numbers of normal cases were the same in both cases.

8.4 Use of other economic Tools

The detection of haemoglobin E is important for the diagnosis of disorders of globin chain synthesis because its interaction with β thalassaemia produces a compound heterozygous state that varies in severity from thalassaemia minor to, more often, thalassaemia intermedia or thalassaemia major. Attempt was made to conduct **Dichlorophenol Iodophenol Precipitation** (DCIP) Test. But the reagents to detect Hb E traits are not available in the country. Moreover, this test is biased towards the diagnosis of Hb Trait rather that thalassaemia as a whole

8.5 Use of Electrophoresis methods.

From literary review it is found that electrophoresis method is most reliable method. Soall tests arecarried out using CE Electrophoresis machine. The test results in the study are based on the results from CE electrophoresis. Hb electrophoresis is being used widely around the world for its confirmatory results of heamoglobinopathy long with its variants.

CHAPTER-9

FINDINGS AND POLICY RECOMMENDATION

9.1 Review Findings

Bangladesh with a current population of about 170 million and a carrier percentage of 10.2 (about 6.1% Hb E and 4.1% Beta-Thalassaemia carriers) has an estimated birth of about 14,500 patients every year.

If we assume that a patient is not diagnosed, which is often the case, and has not received any blood transfusion, the patient will die by the age of 6 years. That means in any year 14,500 patients are being born and 14,500 patients who were born 6 years ago are moving towards death. So it could be assumed that at any time Bangladesh has 6x14,500=87,000 say 90000 living children with Thalassaemia Major or HbE-Beta Thalassaemia. That means 90,000 families are suffering.

A very insignificant number among these 90,000 patients are diagnosed properly. Of those diagnosed, most cannot afford to have treatment as recommended by WHO. Average cost of treatment is between Tk. 7,000 and 20,000 per month (depending severity of the disease and type of iron chelator suited). As a consequence, virtually all 90,000 families are economically and emotionally ruined.

This number is likely to increase with population growth. To minimise suffering of this huge number of patients and their families, Bangladesh should adopt a policy to prevent the disease. Since a patient may be born if both the parents are carriers of Thalassaemia or HbE, it is imperative that each and every one knows about their carrier status and the consequence of giving birth to a thalassaemic child before they get married.

Prevention to be effective requires mass awareness about the disease. The lifelong high cost of treatment may be circulated through TV and other Media. Inclusion of the basics of the disease in primary education may be considered. Whatever may be the preventive policy it cannot be achieved overnight; rather it has to be a continuously run programme. A recent study in Israel on cost of prevention and cost of treatment of a Thalassaemia patient with a life expectancy 50 years, found that prevention cost is about 3.2 % of the treatment cost. Thus, the prevention of affected newborns over a certain period represents a huge saving to the health budget even after deducting the cost of the prevention program.

Policies for prevention of occurrence and subsequent control have been adopted by many countries, where Thalassaemia is a major health problem. Policies adopted in 29 countries with a view to mitigating the problem are stated in Table 9.1. The policies in some countires are similar and have been adopted almost during the same period

Table 9.1: Policies Adopted in Different Countries for Prevention of Thalassaemia

Sl No	Country	Adpoted policy and effects
1	Sri Lanka	**"Safe Marriages" for Thalassaemia Prevention:** “Safe Marriage” that is defined as a marriage where at least one of the partners in a couple is not a carrier for Thalassaemia. It is likely that ths policy will prevent birth of Thalassaemia Patients
2	India	**Preventive steps for prevention of of Thalassaemia in India :** Population education, mass screening of high risk communities for thalassaemia minor, genetic counselling of those who test positive for thalassaemia minor and prenetal diagnosis
3	Pakistan	**Prevention of Thalassaemia in Pakistan**: Have passed laws on thalassaemia prevention proposing compulsory screeing of couples before marriage. Established good number of thalassaeia centres for treatment of patients. Working on developing a national policy to control and eradicate thalassaemia in Paistan
4	Iran	***National Prevention Program:*** Thalassaemia prevention in Iran was initiated in 1995, although it was not until 1997 when mandatory premarital screening was implemented. To control new Thalassaemic births, appropriate strategies have been adopted by the program in which carrier couple screening and genetic counseling to at-risk couples are practiced. It seems that the Thalassaemia prevention program in Iran has fulfilled its goals in many provinces particularly in Thalassaemia affected regions. The prevalence of β-thalassaemia births in Southern Iran decreased by 81.1%, from 2.53/1,000 births in 1995 to 0.82/1,000 births in 2004. The incidence of β-thalassaemia decreased by 96.5% over 10 years in Central Iran, from 43.7/100,000 in 1997 to 1.5/100,000 in 2010 . Iran's successful β-thalassaemia reduction placed the Iranian PMSGC (Pre marital screening and genetic counseling) programme as a benchmark for other national programmes.
5	Iraqi Kurdistan	The PMSGC programme was mandated in 2008 in Kurdistan, Northern Iraq, with prenatal diagnosis (PND) and therapeutic abortion available. In the first 3 years (2008-2010), 91% of at-risk couples proceeded with marriage. PND was sought by 38% of those deciding to marry, and all affected pregnancies were terminated .A 5-year evaluation found that 98% of at-risk couples proceeded with marriage, with PND sought by 76% and 10 of the 11 affected pregnancies terminated. Thirty couples, married prior to 2008 with at least 1 thalassaemia-affected child, were offered PND and all 3 affected foetuses identified were aborted. The number of thalassaemia-affected births in Kurdistan decreased from 20 to 7 over 5 years, a reduction of 65%.
6	Bahrain	The PMSGC programme was mandated in 2004, and PND and therapeutic abortion are legal. A cross-sectional study of 1,070 PMSGC attendees reported a 43.3% at-risk marriage cancellation rate.
7	Jordan	The PMSGC programme was mandated in Jordan in 2004. PND is legal, upon request on a self-pay basis, but therapeutic abortion is

Sl No	Country	Adpoted policy and effects
		illegal. Oseroff found that PMSGC discouraged 40% of at-risk marriages, while the demand for PND was low due to costs and unavailability of abortion. Hamamy and Al-Allawi found that of 48 at-risk couples identified in 2006, only 3 (6%) cancelled marriage plans.
8	Saudi Arabia	The national PMSGC programme, mandated in 2004, offers screening to all couples registered to marry. Couples found to be at risk receive counselling, as PND and therapeutic abortion are illegal [1. Alhamdan etal reported a 10.4% at-risk marriage cancellation rate in 2004-2005. Alswaidi and O'Brien reported a rate of 11.8% for 2005-2006 while Memish and Saeedi found a rate of 26.5% in 2004-2009. An increasing at-risk marriage cancellation trend of 9.2% in 2004 to 51.9% in 2009 was found. Alswaidi and O'Brien reported that carriers with prior knowledge of their status were more likely to cancel marriage with another carrier than those without prior knowledge (11.8 vs. 28.8% cancellation rates, respectively
9	Turkey	PMSGC was mandated in Turkey in the 1990s (in Denizli from 1995 and in Mersin from 1998). PND and therapeutic abortion are legal. A 4-year evaluation in Denizli found a 13.3% marriage cancellation rate among at-risk couples while 40% sought PND, with 1 foetus diagnosed with β-thalassaemia and terminated. Of 135 at-risk pregnancies in 1999-2004, 80 received PND and only 2 receiving PND delivered an affected baby, 1 due to a late referral. A national review indicated that Turkey's PMSGC programme achieved a 90% reduction in β-thalassaemia births annually, reducing from 272 births in 2002 to 25 in 2010. No analysis of changes in β-thalassaemia incidence rates in Turkey was found.
10	Greece	Greece started their prevention program in 1975 and after 35 years the reports indicate significant decrease in Thalassaemia births.
11	Italy, Cyprus, UK, France and Australia	In the late 1970s pilot population programs directed to prevent beta-thalassaemia major by carrier screening, counselling, and prenatal diagnosis among high risk population. At present, the country has set up comprehensive national programs, which include public awareness and education, carrier screening, and counselling, as well as information on prenatal and preimplantation diagnosis.
12	Thailand	In the late 1970s pilot population programs directed to prevent beta-thalassaemia major by carrier screening, counselling, and prenatal diagnosis among high risk population. At present, the country has set up comprehensive national programs, which include public awareness and education, carrier screening, and counselling, as well as information on prenatal and preimplantation diagnosis.
13	Singapore, Taiwan and Hongkong	In the late 1970s pilot population programs directed to prevent beta-thalassaemia major by carrier screening, counselling, and prenatal diagnosis among high risk population. At present, the country has set up comprehensive national programs, which include public awareness and education, carrier screening, and counselling, as well as information on prenatal and preimplantation diagnosis.
14	Cuba	In the late 1970s pilot population programs directed to prevent beta-

Sl No	Country	Adpoted policy and effects
		thalassaemia major by carrier screening, counselling, and prenatal diagnosis among high risk population. At present, the country has set up comprehensive national programs, which include public awareness and education, carrier screening, and counselling, as well as information on prenatal and preimplantation diagnosis.
15	Netherlands, Belgium and Germany	Prenatal diagnosis service has been introduced and partial programs including antenatal screening according to ethnic origin are available
16	Qatar, Lebanon, Tunisia, UAE and Ghaza Strip	The national premarital programs are mandatory and aimed at limiting carrier marriage.Interruption of the pregnancy is permitted for severe fetal disorders but only within the fourth month of gestation
17	China	In China, prenatal screening was compulsory to obtain marriage permission by the marriage law office. Because of worldwide criticism, the requirement for a certificate of premarital screening was withdrawn.

9.2 Survey Findings

9.2.1 Prevalence

The test results of 1439 samples from university college students mainly at Dhaka, Bogra and Noakhali depict that in Bangladesh 10.2% Hb E carrier, 3.7% beta Thalassaemia carrier and about 1% Hb E diseased who do not face problems except under certain conditions.

In Barisal Division in all 10.4% persons were either carriers of Thalassaemia or diseased. Among the carriers 5.9% were carriers of Hb E Haemoglobinopathy while 2.9% were carriers of B Thalassaemia and 2.9% were Hb E diseased.

In Chittagong Division, in all 12.3 % persons were carriers of either form of Thalassaemia prevalent in the country. The Hb E carriers were 8.1 % and others 4.1 %. There is indication that the hill districts have high rate of occurrence.

In Dhaka Division the overall occurrences of Hb E and βThalassaemia carriers are 8.2% and 2.5% respectively. The percentage of Hb E Disease is 0.8 %.

In Khulna Division the overall occurrences of Hb E and β Thalassaemia carriers are 5.0% and 1.3% respectively.

In Rajshahi Division the overall occurrences of Hb E and βThalassaemia carriers are 13.0 % and 2.0% respectively. The percentage of Hb E Disease is 0.1 %. The occurrence Hb E in Rajshahi District is more than 27%

In Rangpur Division the overall occurrences of Hb E and βThalassaemia carriers are 26.7% and 4.6% respectively. The percentage of Hb E Disease is 3.0 %. In Dinajpur District 47.8 % of the people are bearers of Haemoglobinopathy traits

In Sylhet Division the overall occurrences of Hb E and βThalassaemia carriers are 7.1% and 7.1% respectively. The male female distribution shows 12. 8% for females while 15.6% for males.

9.2.2 Patient Characteristics

In all 215 patients/patients guardians were interviewed and record checked. It was found that the occurrence E β Thalassaemia is 49.7% followed by β Thalassaemia by 49.8%. The occurrence of F/β Thalassaemia is only 0.5%. Almost equal number of patients come from both the traits but the number of Hb E trait bearers is almost fo times than that of the β trait bearers.

Most of patients (31.6%) were detected of the disease before the age of six months. Next comes the patients (23.1%) in the age group more than 36 months. 4.7 % patients could not tell the age of first detection.

The most common method for diagnosing affected patients was elctrophoresis. This method is available in only some selected places mostly in Dhaka and one or two other divisional towns. CBC was used in only 5.1% cases, but it gave indicative values for which almost all had to come for electrophorasis for confirmation.

Different symptoms were identified by the guardians of the patients before coming for treatment. Loss of appetite was reported by 61.4% followed by enlarged spleen (51.6%) and improper body growth (48.8%). Jaundice, pale face and Hepatitis, which are almost synonymous was felt by 68.4% of patient's guardians. 1% could not give any response.

Among the guardians of sample patients only 27.9% (31 parents) considered that the operation of spleen to be problematic. Among the patients who underwent spleen operation, 87.1% wastaking penicillin prophylactic medication.

In the country 61.2% of patients are required to transfuse blood 12 times a year or more. 28.5% patients need transfusion 6 – 12 times a year whereas only 10.3% need transfusion less than six times a year.

About 97.6% of patients used packed cell. Among the types washed packed cell, packed cell and packed cell with filter are used by 63.6%, 31.1 and 2.9% patients respectively.

Among the patients having blood transfusion 91.4% informed that iron chelation medicines are taken by them. Most (70.2%) of the patients used Capsule Kelfer (Deferiprone). No medicine is taken by 3.7% of the patients.

About 72.3 % of patients using medicines gave their opinion in respect of side effects. Among them 10.9% said that no side effect was felt by them. Loss of appetite is most commonly felt by 69.9% of the patients followed by pain at joint by 48.7%. The other problems faced are allergy (14.2%) and respiratory problems (7.1%).

The most common problem stated by 45.1 percent of the households are pecuniary. Non availability of treatment facility is reported by 43.7% of patient followed by non availability of safe blood by 39.1 % households. Lack of diagnostic facility was expressed by 16.7% households. No response and others accounted for 3.7% households.

About 45.3% of the households are from the poor having monthly income of less ran Tk. 10000 per month, while 34.9% are from lower middle class having income in the range of Tk 10,000 – 25,000 and remaining 19.8%are in the from upper middle class and above.

Multiple problems were stated by the patients in their day to day life. Intrinsic problems related with health were faced by 22% while social ones were faced by remaining 78 %

9.2.3 Awareness

A pilot survey among 537 households with college and university students was carried out with responses from the students. It is found that 51.2% did not know what type of disease it is. 39.3% knew that it was a non-contagious disease (NCD). About 8.9% of the respondents thought it to be contagious and 0.6 % considered it to be psychological disorder.

Among those (39.3%), who considered it as NCD 6.16 % could not tell what type of type it was. Among them 4.27 % this considered to be another type of disease. However, 89.6 % of them could tell that it was genetic disease. Thus among all respondents only 35.2%, 2.4% and 1.7% respectively considered it as genetic, infectious and other type of disease.

The source of knowledge of 34.1% household members was family and friends followed by social media (25.1%). The print media accounts for the awareness of about one fifth of the persons. Knowledge from medical professionals is only 2.4%.

The discussion with 215 patient guardians/ Patients reveals that more than 85% of the patient households understood that Thalassaemia is a non contagious genetic disease. They rather have a clear idea about the disease. Another 6.5% understands that it is non contagious but its genetic role is not understood. About 0.5 % of the households still consider it to be contagious disease. About 8% of these households consider this to be physical or other type of problems.

In spite of the close interaction with hospital and doctors only 63.7 % of the guardians know that it occurs only when both the parents are Thalassaemia trait bearers. About 7 % consider that occurs like other diseases and have no relevance with the Thalassaemaic blood characteristic of the parents. About one third (29.3%) households have no clear idea how the disease occurs.

About 94.4% of patient households understood the problem. They informed that different symptoms occur and fever is the most common of these.

The community as a whole is not aware of the problem. But once appraised they gave suggestions for facing the problem.

9.2.4 Screening Tool Used

CE electrophoresis, NESTROFT and CBC methods were tested. NESTROFT method could not identify Hb E. CBC Method could identify the blood disorder properly but it could not distinguish between Hb E and Beta Thalassaemia. CE electrophoresis could properly identify the differences. But the instrument is available in Dhaka and one or two other places in the country. The study identified the situation of country on a pilot basis and gave indicative result. A study with appropriate statis tical sampling covering the whole country is needed to find the actual situation.

9.3 Policy Recommended for Bangladesh

In most of the countries where Thalassaemia disease is a major public health the following are concerns to be considered:

- National programmes for its control do not exist.
- Basic facilities to manage patients are absent,
- Screening for thalassaemia disease is not a common practice
- The diagnosis of the disease is made when severe complication occurs.

9.3.1 Specific Recommendation on Tools

Though NESTROFT and DCIP are less expensive tools for screening Beta thalassaemia and Hb E traits; however they are prone to be erroneous. CBC screens iron deficiency anemia or anemia due to some genetic heamoglobin disorder. Hb electrophoresis by Capilary electrophoresis (CE) or HPLC detect the genetic heamoglobin disorder trait as well as disease with confirmation.

NESTROFT + DCIP + Hb electrophoresis will be very cumbersome process to control where as CBC will not identify Hb-E.

Under the circumstances the study suggests to use Hb electrophoresis as a main tool for mass screening.

In our opinion, a carrier detection procedure should be designed to avoid missing any couple at risk. For this reason we have included in the first group of examination, in addition to determination of MCV–MCH, the quantitative evaluation of HbA_2, which may be obtained by electrophoresis or by high-pressure liquid chromatography (HPLC). HPLC has the additional advantage to quantitate also HbF and to detect clinical relevant Hb variants including Hb Knossos (a mild β-thalassemia allele), HbS, HbC, HbD Punjab, HbO Arab and HbE, all of which may interact with β-thalassemia heterozygosity leading to thalassemia major or intermedia or sickle cell β-thalassemia

9.4 General Recommendations

1. A broad based screening is to be undertaken through out the country based on scientific statistical sampling method to identify the prevalence trait bearers. (Approximately a sample size of around 25,000 may suffice)
2. Premarital Screening Programme: All people getting married should be screened before their marriage is solemnized. Accordly a law for Safe Marriage is to be enacted.
3. Setting up Thalassaemial screening and genetic counseling programmes: The disease should be identified during the prenatal period or at birth as part of a routine screening programme. Genetic counseling and screening can lead to reduction in the number of children born with the trait. The programme should be developed at the primary care level with appropriate referral system.
4. Parents must be counseled to seek medical care for all febrile events in children with Thalassaemia diseases.
5. Training of health personnel in prevention, diagnosis and case management should be an integral part of the national programme.
6. Integration of national control program for Thalassaemia disease within the national programmes for prevention & control of non communicable disease (like Cancer, Diabetes).
7. Setting up the antenatal screening programm: Antenatal tests shall be carried out on pregnant women, who are known carriers and whose spouses are also carrier for the trait, subject to approval having been obtained from the pregnant women and their spouses.
8. Setting up prenatal screening programme: To Identify women/couples at risk of a pregnancy with thalassaemia disorders and provide appropriate referral & care for prenatal diagnosis with continuation or termination of pregnancy according to family's choices.
9. Vigorous awareness campaign (like family planning, immunization, etc.) on the prognosis of thalassaemia disease through print media, mobile network, electronic media and through distribution of leaflet, booklets poeters, etc is to be carried out,
10. Inclusion of information on Heamoglobanopathies in school curriculum for continuous dessimination among students.
11. Preparation of central digital Thalassaemia patient database and issue identity card to ensure privilege of getting treatment support in public and private health centers.

Appendix

Appendix-1: Questionnaire for Sample Interviewees (Quantitative survey)

Appendix-2: Checklist for Key Informant's Interview

Appendix-3: Checklist for Focus Group Discussion (FGD) with Stake Holders

Appendix-4: Semi Structured Questionnaire (Patient and Parents)

Appendix-5: Carrier/Diseases Detection Registration From

Appendix-6: Electrophoresis Report Samples

Appendix-7: References

Appendix-8: References

Appendix - 1

Questionnaire for Sample Interviewees (Quantitative survey)

Personal Information:

01. Name:…… Age: Sex: male /female
02. Father's name: .....
03. Mother's name: …….....
04. Marital status: married / unmarried 5. Occupation:

06 Present Address (Present):

House: …….. Road: ……..... Block: …… …
Village: …… Thana: …… Post Code: …….....
District: ……

07 Permanent Address :

Holding No/ House: …… Village: ……
Post code -------- Upazila: …….. District:

Questions on Thalassaemia

08. Do you know that Thalassaemia is a disease? Yes/No (Tick, if No go to 14)
09. What type of disease is it? Contagious (1) Non contagious (2) Psychological (3)

    If 2 and 3 go to 14
10. If non contagious what type is it? Genetic/ Inherited (1), infectious (2), others (3)
11. How did you come to know about Thalassaemia? Social media (1), Print media(2), Family and friends (3) Personal doctor (4) others (5)
12. Are you a Thalassaemia patient? : Yes (1) No (2) If yes
13. What are the symptoms? Note
14. Do you think Thalassaemia can spread? Yes (i) No (ii) (Tick), if No go to 17
15. How does this disease spread? Contacts with Thalassaemia carrier (1), Marriage between two Thalasseamia carriers (2), Family (3) Do not know (4)
16. How can the spread be prevented? Preventing marriage between carriers (1), By treatment (2), Cannot be prevented (3)
17. How can you detect a Thalasseamia carrier?

    By normal blood test (1), By special blood test for thalassaemia (2) None (3)
18. Are you Thalasseamia carrier? Yes (1) No (2), If No go to 21
19. When did you learn that you are a Thalassaemia Carrier? After symptoms (1), Test before marriage (2), At the start of pregnancy (3)
20. How did you learn that you are a carrier of Thalasseamia? By normal blood test (1), Thalassaemia blood test (2) others (3)
21. Do you know the percentage of population, which is Thalassaemia career? Yes (1) No (2) If no go to 23

22. If yes what is the approximate value?

23. If one learns before marriage that one and ones proposed partner is a Thalasseamia carriers than marriage should be stop? Yes(1), no (2), Do not know (3)

24. Do you think not having or abortion is a way to prevent Thalassaemia? Yes (1) No(2) abortion after test (3) others (4)

25. Do you know Thalasseamia requires costly treatment and Transfusion of blood? (yes (1) No (2)

26. Is the government action in this respect is adequate. Yes (1) no (1)

27. What the civil society should do in preventing spread of Thalasseamia?

    Awareness creation (1) Organizing occasions to spread knowledge (2)Invoke government action (3)

28. What action should the government take to prevent spread of Thalassaemia?

    Screen all effective people (i)/ Include in national ID card Thalasseamia status along with blood group (ii)/ Enact necessary law (iii) Take national programme of screening (iv) /develop national programme for preventing spread of it (v)

Thank you for your patient hearing and responses. You will be given a test report.

Appendix 2

Checklist for Key Informant's Interview

1. Thalasseamia is a serious health problem but not understood by the society. How the problem may be addressed by the people.

2. The support to the Thalasseamia patients is very meagre. How should it be addressed.

3. The cost of treatment is high and blood requirement is also difficult to meet. What should be the steps?

4. As a Specialist, you know prevention is better than cure. What should be the programme so that within a time frame the occurrence of this disease is prevented.

5. Should the government enact a law in this respect that all eligible aged persons undergo screening?

6. Do you think it will be practicable to introduce Thalasseamia status along with blood group in national ID Card.

7. According to your opinion which diagnostic method is reliable and cost effective for detecting Thalassamea How an economical method may be taken up so that the eligible population in country may be screened.

8. Do you think the medical infrastructure is sufficient to undertake the task in a short time or a perspective plan is to be developed.

9. What should be the time frame and give the logic.

10. Until a total preventive step is there, what support should the government give to the Thalasseamia patients

11. Any other specific Suggestion and opinion?

Appendix -3

Checklist for Focus Group Discussion (FGD) with Stake Holders

1. Thalasseamia is a serious health problem what are you opinions please discuss.

(If all are ignorant or little knowledgeable discuss in brief the problem and give pamphlets)

2. As you now understand it is a genetically inherited disease. What the civil society should do in preventing the spread of the disease.

3. Should a national screening program be taken up and how the people may be provided authentic document about the thalasseamia status of a person?

4. The present screening process involves both manual and automatic process. The manual process has the scope of error in detection but is relatively cheap. But the electrophorasis process, which is automatic and costly gives error free results. Discuss and give your opinion on the method of detection.

5. What should be government role in this respect. Should a law be enacted. Please discuss.

6. Law alone cannot achieve much; should the government take up a short term or perspective plan for preventing the problem.

7. Should the Thalasseamia characteristic be included along with the blood group in the national ID card. Do you think the individuals will like it. In spite of dislike should this be incorporated. The ID card form would also need modification. Would the authority agree. Could the members of the parliament may be motivated in this regard. Discuss point by point ?

8. Until the process is finalized the support to the patients should continue. What should be government role. What the civil society should do?

9. Is insurance policy support viable for patients.

10. Do you think the medical infrastructure is sufficient to undertake the task in a short time or a perspective plan is to be developed.

11. Any other specific Suggestion and opinion

Appendix -4

Semi Structured Questionnaire (Patient and Parents)

Date of Interview:
Registration No.or Code No. :
Personal Information:

05. Name of the Interviewee:…… Sex: male /female
    Age: Education:
    Contact No: Weight:

06. Father's name:
    Age:.....

07. Mother's name:
    Age:…….....

08. Marital status: married / unmarried 5. Occupation:

08 Present Address (Present):

House: …….. Road: ……..... Block: …… …
Village: …… Thana: …… Post Code: ……....
District: ……

09 Permanent Address :

Holding No/ House: ……. Village: ……
Post code -------- Upazila: …….. District:

08. When was the disease diagnosed (at what age)?
    Below 1 yr(1) ; yr1 –yr 10(2): Yr10 – yr 203(); 1bove 20(4)

09. Have your parents been tested for thalassaemia?

10. Do you know that consanguinity has a role in thalassaemia.

11. What symptoms/ problems were faced before identification? Note down and code

Code: According to Physical Change (1), Physical activity (2) Clinical features (3)

12. How you were diagnosed as a Thalassaemia patient? Note

13. How much has this disease affected your normal life routine?
Physical(1); Menta(2); both(3); others(4)

14. How do you feel about yourself in the society? Note and post code

Code: People look at us a low profile (1), Friendlessness (2), Uncertainty in future (3), Uncertainty in marriage (4), Low achievement in academic arena (5) ; Social burden(6); others(7)

15. What is the monthly household income of yours/your family?

Below Tk 10000(1), Tk 10000- Tk 25000(2), Tk25000-50000(3), Above Tk 50000(4)

16. What are Effects of disease on your economic status.

17. What is the Effects of disease on family size.

18. What are your problems in relation to the treatment? Note and post code

Code: Lack of medical support in the vicinity (1), Dearth of blood for transfusion (2), Lack of diagnostic facility in the vicinity (3) Dearth of money (4) Others, Specify (5)

19 Do you think not having or abortion is a way to prevent Thalassaemia?
Yes (1) No(2) abortion after test (3) others (4)

20 How can we prevent thalassaemia.

CRDS-BTS JOINT VENTURE
(থ্যালাসেমিয়া বিষয়ক একটি প্রকল্প)
Appendix - 5

SI.NO :

Date :

থ্যালাসেমিয়া বাহক/রোগ নির্ণয় নিবন্ধন পত্র

১. Name (English): ……..

নাম (বাংলায়) : .....

২. বয়স:……..…….....……

৩. ব্লাড গ্রুপ :.....

৪. Father's Name(English) :.....

বাংলায় :……..……..…..……

৫. Mother's Name (English) : .....

বাংলায় : .....

৬. লিঙ্গ :….. ছেলে/মেয়ে ৫. বৈবাহিক অবস্থা :..... বিবাহিত/অবিবাহিত

৭. পেশা : ছাত্র/ছাত্রী/চাকুরী/ব্যবসায়ী/অন্যান্য

৮. বর্তমান ঠিকানা :.....

জেলা :.....

৯. স্থায়ী ঠিকানা :.....

জেলা :.....

১০. মোবাইল নং :..... ই-মেইল : .....

১১. জাতীয় পরিচয়পত্র নং : .....

১২. ডায়াগনোসিস :

Result	Comment

স্বাক্ষর

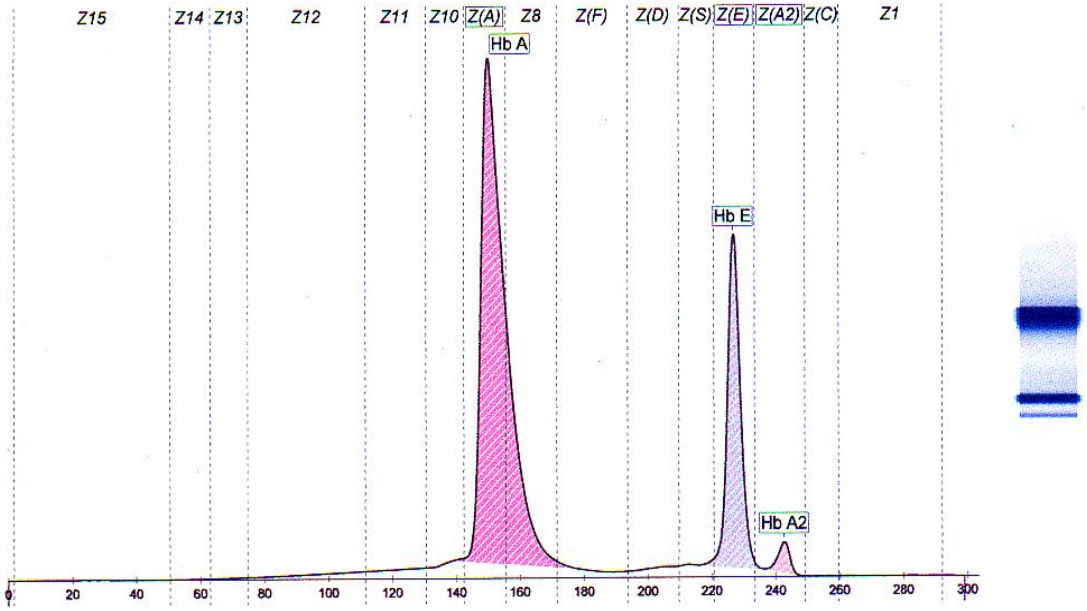
প্রতি স্বাক্ষর

Appendix-6

Electrophoresis Report Samples

Sample-1

Estimation carried out by Sebia Fully Automated Minicap flex piercing



Haemoglobin Electrophoresis

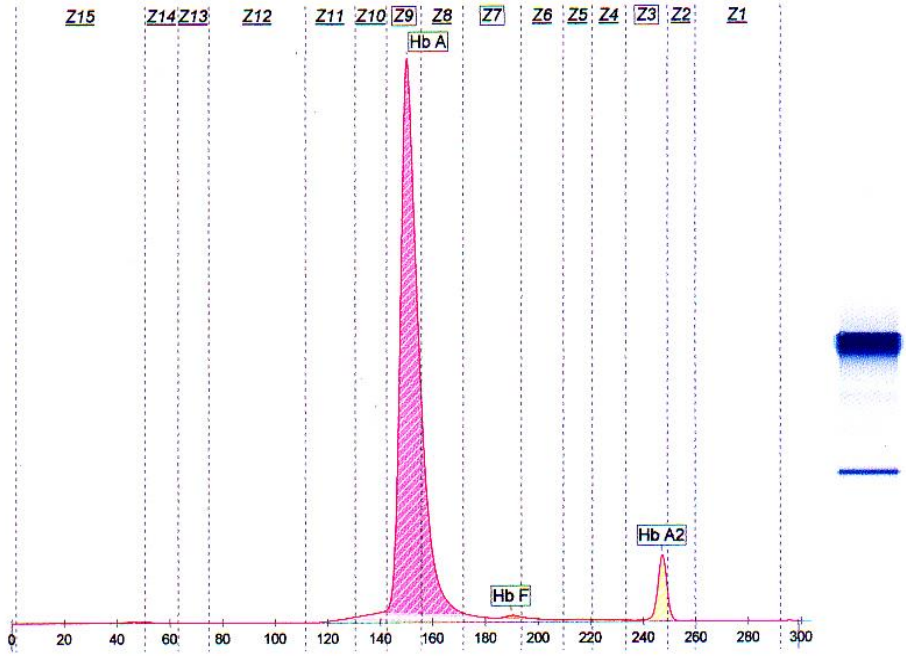
Name	%	Normal Values %
Hb A	72.6	96.8 - 97.8
Hb E	25.3	
Hb A2	2.1	2.2 - 3.2

Comments: Haemoglobin E Trait

Advice : Genetic Counselling

Sample-2

Estimation carried out by Sebia Fully Automated Capillarys 2 System



Haemoglobin Electrophoresis

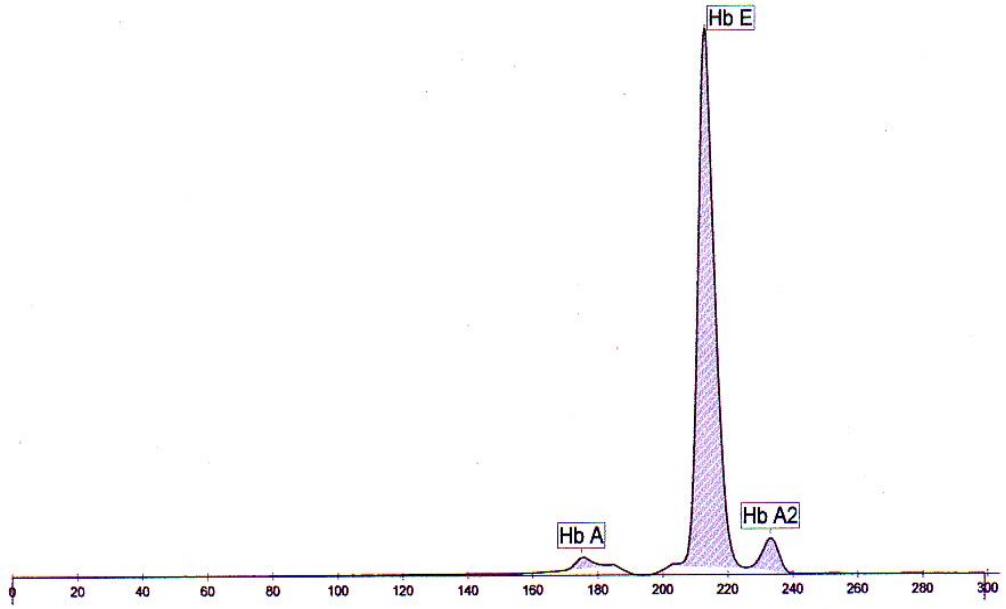
Fractions	%	Normal Values %
Hb A	93.8	96.8 - 97.8
Hb F	0.5	0.0 - 1.0
Hb A2	5.7	2.2 - 3.2

Comments : Beta Thalassaemia Carrier

Advice : Genetic Counselling

Sample-3

Estimation carried out by Sebia Fully Automated Minicap flex piercing



Haemoglobin Electrophoresis

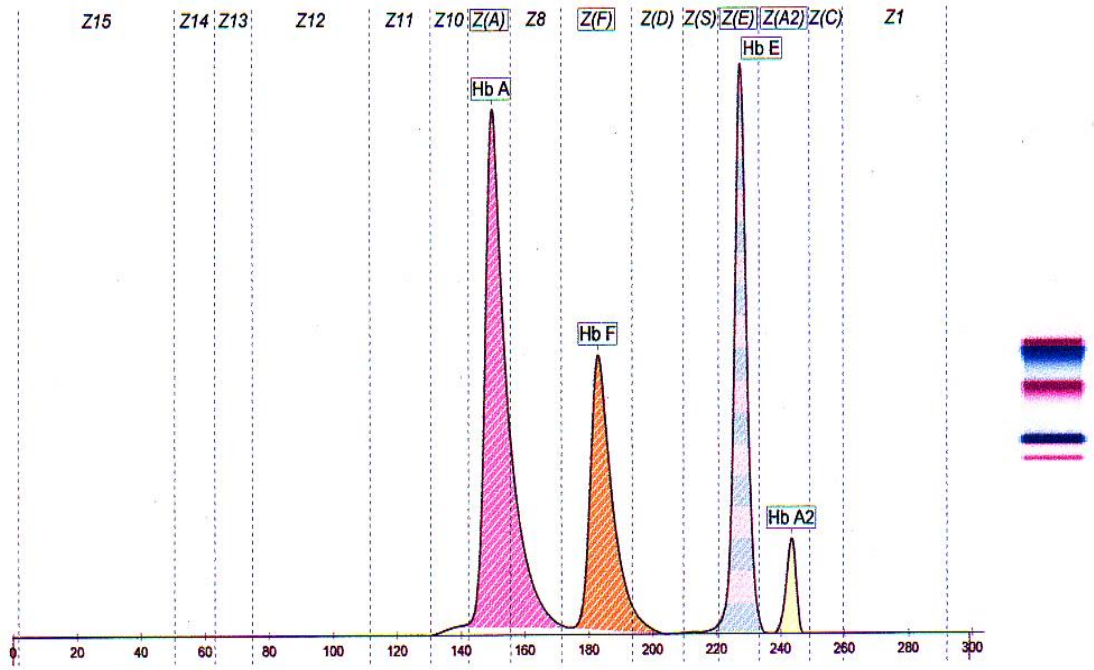
Name	%	Normal Values %
Hb A	1.8	96.8 - 97.8
Hb E	92.8	
Hb A2	5.4	2.2 - 3.2

Comments: Haemoglobin E Disease

Advice : Genetic Counselling

Sample-4

Estimation carried out by Sebia Fully Automated Minicap flex piercing



Haemoglobin Electrophoresis

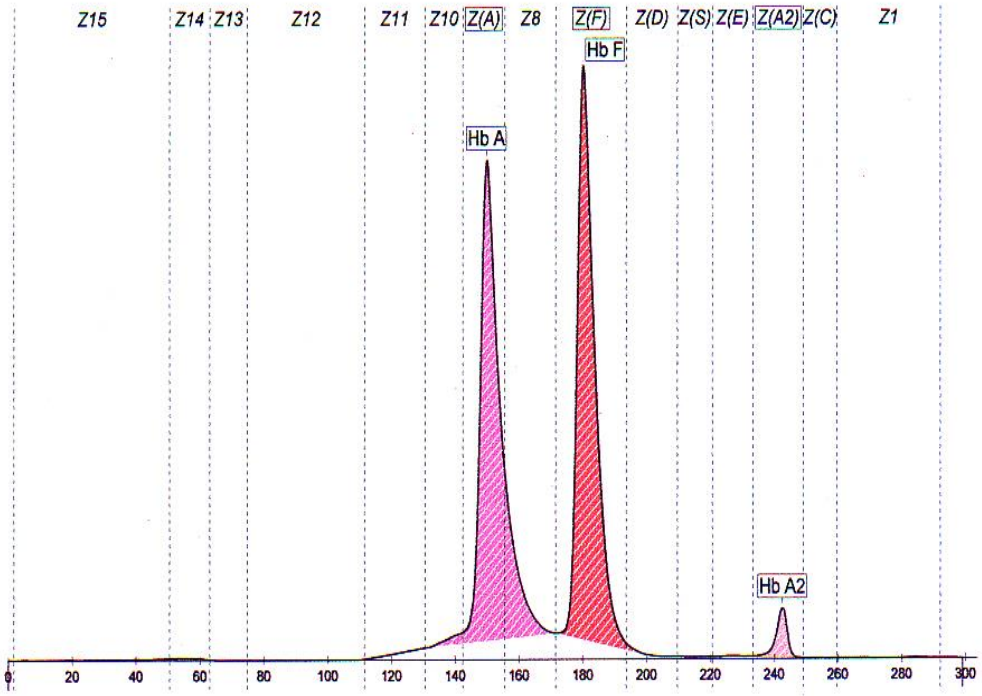
Name	%	Normal Values %
Hb A	44.8	96.8 – 97.8
Hb F	23.6	0.0 – 1
Hb E	27.9	
Hb A2	3.7	2.2 – 3.2

Comments: Haemoglobin E Beta Thalassaemia

Advice : Genetic Counselling

Sample-5

Estimation carried out by Sebia Fully Automated Minicap flex piercing



Haemoglobin Electrophoresis

Name	%	Normal Values %
Hb A	**48.7**	96.8 - 97.8
Hb F	**48.7**	=< 0.5
Hb A2	**2.6**	2.2 - 3.2

Comments: Beta Thalassaemia

Advice : Genetic Counselling

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