THALASSAEMIA INTERNATIONAL FEDERATION

DELEGATION VISIT TO BANGLADESH

28th January to 1st February 2019

Report of a delegation visit to Bangladesh, supported by Thalassaemia International Federation, the Ministry of Health and Bangladesh Thalassaemia Samity Hospital

REPORT

BANGLADESH
THALASSAEMIA
SAMITY

FOREWORD

Bangladesh Thalassemia Samity (BTS) is a private non-government organization. Its members are thalassaemia patients or parent(s) of thalassaemic children.

BTS has been around for almost three decades. Its main activity was giving treatment to its member and non-member patients according to WHO and TIF protocol, create public awareness and ultimately prevent of diseased child.

In the final few day of January, to be precise January 31st of February 2nd TIF and BTS jointly held a workshop, whish was participated by Ministry Of Health trough DGHS. Many physicians attended the work-shop.

TIF has prepared a report on the workshop based on the deliberations and feedback received by its panel of experts.

The report is presented with the hope that it will help the Ministry to formulate a National Policy for Management, Treatment and Prevention of Thalassaema.

Omar Golam Rabbany
President
Bangldesh Thalassaemia Samity

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1. Purpose of visit:

1. To participate in an international workshop on management and prevention of thalassaemia, organised by the Ministry of Health in Bangladesh.

2. To discuss the current situation and plans for development of thalassaemia awareness, prevention and care programmes in Bangladesh.

3. To review treatment facilities for patients

4. To develop future collaborations to support these initiative

2. International delegates

- Dr Paul Telfer. Consultant Paediatric and Adult Haematologist, Barts Health NHS Trust, Senior Lecturer in Haematology, Queen Mary University of London, UK
- Dr Mary Petrou, Consultant Clinical Molecular Geneticist, University College Hospital NHS Foundation Trust, Senior Lecturer University College London
- Professor Maitreyee Bhattacharyya, Director, IHTM, Kolkata, In charge , Nodal Centre for State Thalassaemia Control Program West Bengal
- Dr Amin Islam, Consultant Haematologist, Southend University Hospital, UK
- Professor Anne Dalton, Genomics, University of Sheffield, UK and Health innovation challenge fund, Department of Health UK

3. BACKGROUND

3.1. Demographics

Bangladesh has a population of 161 million, occupying 147 570 square kilometres. About 90% of the population are Muslims, 9% Hindus and 1% Christians, Buddists and other faiths. Bangladesh has experienced a remarkable rapid decline in fertility rate from 6.9 births per woman in the early 1970's to 2.1. Administratively the country is divided into seven divisions, 64 districts and 545 subdistricts (upazila).

3.2 Health Status

Bangladesh is a low income country. During the last forty years since independence Bangladesh has made remarkable improvements with life expectancy, child health and literacy.

Especially during the last 15-20 years GDP has increased steadily and during the past 5 years has increased by almost 8% per annum. Health indices are also very encouraging showing a steady decrease in under 5 mortality from >140 per 1000 live births in 1990 to about 30 per 1000 live births in 2018. These have been achieved by improvements in key interventions, such as a delivery of a health facility, childhood immunisations and management of diarrhoea with oral rehydration salts and treatment of TB.

However, the provision of services for the growing burden of non-communicable diseases such as the thalassaemia disorders is likely just beginning. We have seen that the quality of care is mostly poor, attributed partly to the lack of safe and adequate blood, an inadequate supply of iron chelating drugs and monitoring of complication by specialists. There is little assessment of the quality of provider care and some low levels of professional knowledge. But we were also greatly encouraged to meet some very motivated health professionals doing their very best to treat the patients and provide a basic testing facilities for patients.

The Bangladeshi Ministry of Health acknowledges that thalassaemia is a common inherited condition in the Bangladeshi population and an important cause of chronic ill health and early mortality. The ministry is committed to planning and implementing a thalassaemia programme, but has not yet confirmed funding plans.

Bangladesh has an extensible Primary Health Care system and the Ministry of Health and Family Welfare, through the Directorates Directorate General of Health Services (DGHS) and Family Planning (DGFP) manages a dual system of general Health and family planning services through:

- 53 District hospitals
- 425 Upazila Health complexes
- 1469 Union Health and Family Welfare Centres
- 12248 Community Clinics
- There are also urban care services managed by Ministry of local government, however I

was informed the quality of services is quite low.
To facilitate research and training in medical science, there are a number of institutuions under the Ministry of Health. There are 21 government medical colleges, six postgraduate institutes, three specialised institutes, two institutes for health technology and five medical assistant training schools. For research there is the Bangladesh Medical Research Council (BMRC) and the National Institute of Population Research (NIPORT)

3.3 NGO's

To compliment the limited capacity and resources of basic health services, the private sector and large NGO health providers, are present in Bangladesh and have a network providing basic health services. We met four such organisations in Dhaka, Bangladesh Samity Thalassaemia Hospital, Youth Club of Bangladesh, Lab One Foundation of Thalassaemia (LOFT) and Biomedical Research Foundation Bangladesh which are actively providing thalassaemia services. The most notable is the Bangladesh Samity Hospital that provides subsidised treatment to 3500 registered patients with HbE/beta thalassaemia and Beta thalassaemia major and transfuses 30-35 patients daily.

3.4. Diagnostics

Along with private clinics and hospitals there are also a large number of private laboratories

3.5. Economic Burden

Thalassaemia places a huge financial burden on the families as the majority of the treatment is self-funded. It is clear that if thalassaemia is to be controlled that a prevention policy needs to be implemented in Bangladesh, as well as the adoption in the future of a universal health coverage policy.

3.6. Epidemiology of the Haemoglobin Disorders

Thalassaemia is the commonest genetic condition affecting the Bangladeshi population. The clinically significant abnormalities are beta thalassaemia (carrier frequency 1-5%) and haemoglobin E (carrier frequency 4-10%). There is no national registry and estimates are of 30-50,000 affected patients in Bangladesh.

A review of existing literature was carried out prior to the visit to gain an insight the scale of the thalassaemia births in Bangladesh. Reviewed documents included the DGHS Report on Non-Communicable Diseases Control Programme, Mohakhali, Dhaka- 1212 (Survey for Determination of Prevalence of Thalassaemia and Launching of Economic Screening Tool to Adopt in National Health Policy to Prevent Thalassaemia in Bangladesh). Other literature review included published papers on screening at the Bangladesh Samity Hospital, Professor Waqar Khan surveys in school children and Tribal groups, data from Rajshahi et al.

Table 1. Carrier frequencies in studies of different populations amalgamated from several sources from the DGHS report.

Division	Number tested	Heterozygote β Thal Number (%)	AE	EE
Barisal	77	2 (2.6)	4 (5.2)	2 (2.6)
Chittagong	260	17 (6.5)	20 (7.7)	1 (0.4)
Dhaka	475	12 (2.5)	39 (8.2)	4 (0.8)
Khulna	159	2 (1.3)	8 (5.0)	0 (0.0)
Rajshahi	338	14 (4.1)	44 (13.0)	0 (0.0)
Rangpur	116	5 (4.3)	31 (26.7)	7 (6.0)
Sylhet	14	1 (7.1)	1 (7.1)	0 (0)
Total	**1439**	**53 (3.7)**	**147 (10.2)**	**14 (1.0)**

The DGHS report gives a carrier frequency of 4.1% for beta thalassaemia and 6.1% for HbAE but Table 1 shows the HbAE carrier frequency is higher. Further surveys in the tribal populations by Prof Khan and colleagues has shown a carrier frequency for HbAE of 35.7% and recently by Bangladesh Samity Hospital (personal communication Omar Rabanny) has shown a carrier frequency for HbAE 40% in Tribal students in the Chittagong area (near Myanmar). This data for was used for the epidemiological analysis below shown in Figures 1-4.

Using the demographic data of Bangladesh and gene frequency, Hardy- Weinberg equation estimates of the annual births of haemoglobin variants by genotype were made. It is estimated that least 9250-10.000 children are born annually in Bangladesh with pathological haemoglobin disorders: 7503 HbE/beta thalassaemia (EBT) (BTM), 1725 beta thalassaemia major with the majority receiving inadequate treatment. 6800 children are born with **HbEE which does not require medical therapy or follow-up.

**It is important for HbEE not to be referred to as HbEE disease.

It is estimated (Figure 4) that there are 6900 at risk pregnancies for homozygous beta thalassaemia and 30.000 at risk pregnancies for EBT, therefore a total 36.900 at risk pregnancies. Table 2 shows an estimate of the number of screening tests that would be required annually for a screening policy that tests the male partner first.

These estimates therefore emphasize the need for a prevention programme for both EBT and BTM, and

the need for an evidence based, effective and feasible management protocol for EBT distinct from beta thalassaemia major

Figure 1. Bangladesh 2015. Distribution (%) of the population by Administrative Division, arranged north to south.

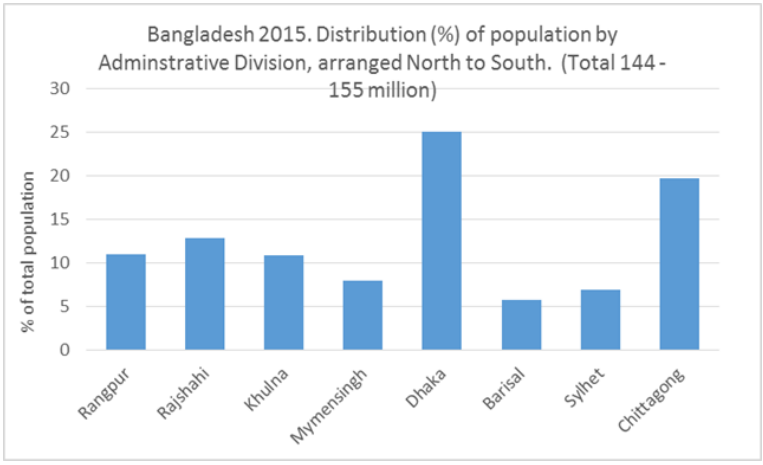


Figure 2. Prevalence of carriers of thalassaemia by Administrative Division

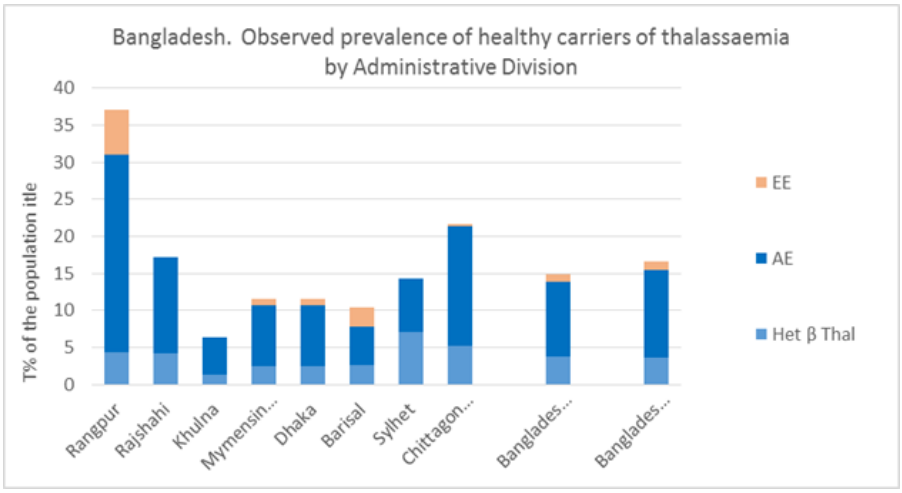


Figure 3. Estimated annual births of homozygous and compound heterozygotes by Administrative Division

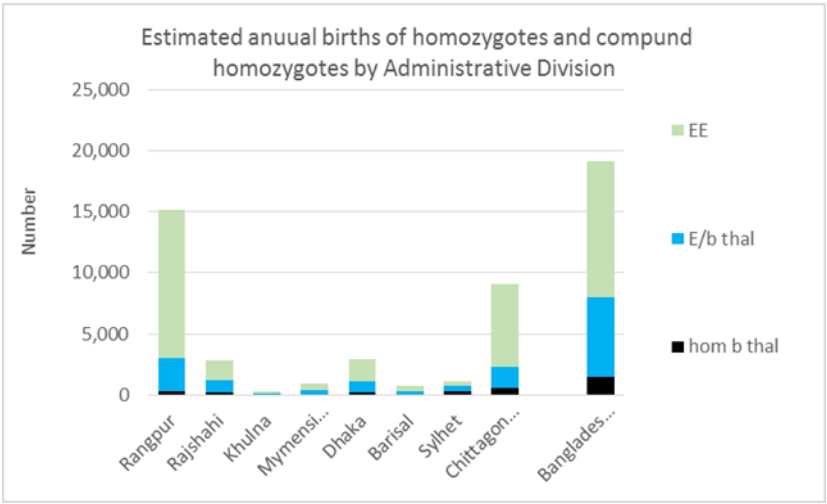
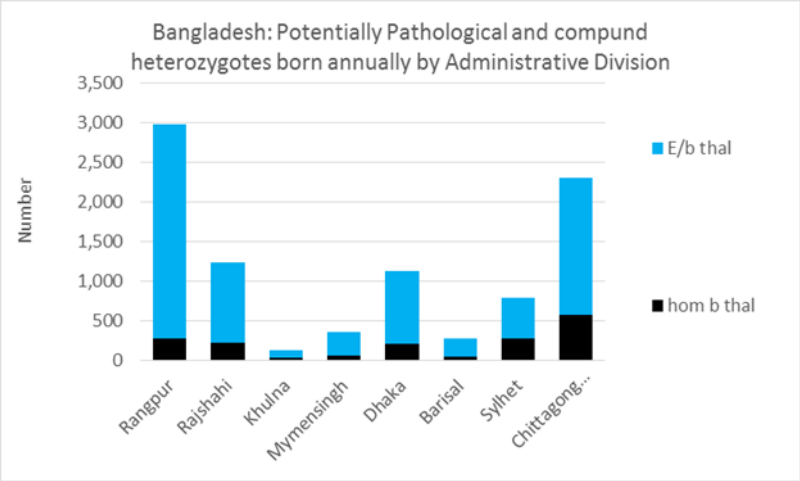


Figure 4. Potentially Pathological and compound heterozygotes born annually by Administrative Division



3.7 Findings

The commonest thalassaemia syndrome, EBT is variable in clinical severity, about 20-30% require regular transfusions from early childhood (phenotypically thalassaemia major) another 50% are moderate to severe thalassaemia intermedia: symptomatic, but requiring only occasional transfusion in childhood. The remaining 25% are mild thalassaemia intermedia, and may not be diagnosed at all during childhood. This high prevalence of HbE/beta thalassaemia in Bangladesh is also a feature of thalassaemia epidemiology in E India, (Calcutta district) and in SE Asian countries including Thailand.

There is no prevention programme in Bangladesh. There is no indication that carrier screening is offered at the premarital stage or to pregnant women.

4. THALASSAEMIA INTERNATIONAL WORKSHOP

Monday 28th January-Thursday 31st January

At the opening ceremony of the international workshop, there were inspiring presentations from Prof. Dr. Be-Nazir Ahmed, Sr. National Consultant, HSS, UNICEF & CSC, DGHS and Dr Nur Mohammed, Line Director NCDC, Directorate of General Health Care Services.
There are already good examples of developments in public health in Bangladesh. For thalassaemia, they highlighted the requirement for a strategy for public communication and awareness of the disease, prevention, access to care and improved management of thalassaemia. They drew attention to examples of prevention programmes in other countries, and stated a desire for a thalassaemia-free Bangladesh by 2030.
The hope was for the clinicians attending the workshop to transform attitude and practice. The international experts attending the workshop and others would be welcome to create fruitful international collaborations for the future.

In keeping with the plan for regional tertiary medical centres, the overall national plan is for 8 thalassaemia centres. At present there are no specialist centres outside of Dhaka, and these are needed to implement a national care and prevention programme. The requirement for national standard guidelines based on existing international standards was emphasized. The draft national guideline was to be discussed at the workshop. Treatment centres would be linked with existing medical colleges. For these activities, an appropriate budget is being evaluated, but not yet agreed.

The draft national guidelines for diagnosis and treatment of thalassaemia have been prepared by a panel of doctors and scientists from the haematology, paediatrics and thalassaemia services. These were discussed with the international delegation and suggestions made for editing and modification. A section on management of Haemoglobin E beta thalassaemia is needed. International delegates and TIF

representatives will be happy to review the next version of the guidelines and make further suggestions prior to implementation

- Drs Telfer and Petrou gave presentations on international standards and examples for diagnosis and management of BTM and EBT.

- Dr Bhattacharya presented her experience with diagnosis and management from the perspective of a very large academic thalassaemia centre in Calcutta. The demography and management challenges are rather similar in E India to Bangladesh, and these presentations were especially useful to the delegates, and delivered in the shared native language of Bengali.

On Day 2 of the workshop the delegates were medical practitioners and on Day 3 they were laboratory scientists, doctors and researchers.

- The difficulties in raising public awareness, and implementing prevention and treatment programmes in a low income country are well known. Public education is needed to dispel the stigma associated with chronic disease and to raise public understanding of the consequences of a carrier diagnosis. Some insight into the level of awareness and attitude towards thalassaemia was provided by a questionnaire study of secondary school pupils in a town within 50 kilometres of Dhaka. This was done by a group of researchers lead by Mohammad Sorowar Hossain, Enayetur Raheem, Md. Mahbub Hasan, Abdullah Al Mosabbir Muhammad Sougatul Islam, Mahbubul H. Siddiqee from Biomedical Research Foundation, Dhaka, Bangladesh, Independent University, Bangladesh (IUB), Dhaka, Bangladesh University of Health Sciences, Dhaka, University of Chittagong, BRAC University, Dhaka. Members of the group attended the workshop and made a presentation to the international experts during the visit.

Action: A paper presenting the results of this work have been submitted for publication in a peer reviewed journal. This and other work of the group will be very helpful in developing a case and for planning strategy for a public awareness strategy and screening programme. The international delegates and TIF would be happy to become further involved in this work.
Regarding treatment for thalassaemia major, the current situation is that the majority of infants are neither diagnosed nor treated, and die in early infancy from consequences of anaemia. It is still the case that organised treatment is only available at the thalassaemia clinics in Dhaka, and the costs of treatment, even when discounted, are beyond the means of the large majority of families.

- Dr Islam provided a poignant illustration of this from his own experience whilst visiting the Sylet region recently. He was put in contact with the father of a sick infant, who had previously lost another child at a very young age with a similar, undiagnosed illness. Dr Islam saw the infant and was quickly able to diagnose thalassamia on account of the pallor and thalassaemic facial changes. The father had never heard of thalassaemia. Dr Islam offered money for the father to go to Dhaka for full diagnosis and for transfusion. The father thanked him and took the money.

- Subsequently he contacted Dr Islam to request using the money to buy clothes for the family rather than for treating the child. The journey to Dhaka and cost of transfusion would keep the child alive for a few months until the next transfusion was needed, at which stage, the family would not be able to afford any further transfusions. This is likely to be a very common, but generally unreported scenario in Bangladesh.

For EBT, the management of those with a moderate or severe phenotype is problematic even in countries with well-developed health care systems. There are compelling reasons to treat these patients in the same way as thalassaemia major- regular transfusion and chelation from an early age to prevent long-term ill health and irreversible organ damage. Unfortunately, safe blood and iron chelation drugs are only available for those treated in the Dhaka thalassaemia clinics, and only for those with substantial financial means, perhaps 1% of the thalassaemia population. Without treatment, children will develop massive splenomegaly, thalassaemic bone changes and extramedullary haematopoiesis. Growth and

pubertal development will be impaired and quality of life significantly restricted because of chronic anaemia leading to death probably in the third or fourth decade. Hydroxyurea has a role in treatment of EBT, but the effect is relatively modest. There is usually a rise in haemoglobin of about 1g/dl, but escalation of dose results in pancytopenia. Splenectomy is probably best avoided because of the risks of overwhelming infection (There were reports of several children at DSH and the Bangladesh Thalassaemia society clinic dying of severe infection within a few months or years of splenectomy). Inadequate transfusion results in the unfortunate combination of symptomatic anaemia, splenomegaly. extramedullary haematopoiesis, and transfusion-related iron overload.

This latter situation was observed in several of the children being treated at DSH. This treatment dilemma has not been resolved and requires international efforts to identify risk groups, observational and controlled trials of different therapy outcomes in a low income setting. There may be a role for new non-transfusion therapies such as Luspatercept.

Action: Further clinical data is needed to guide management recommendations for EBT.

The following would be valuable

1. Long -term outcomes and natural history of EBT in Bangladesh

2. Retrospective and prospective study of benefits and risks of splenectomy
3. Role of hydroxyurea (HU) in transfusion avoidance in EBT. Possibly a randomised controlled trial comparing HU with transfusion

4. Collaboration with Celgene and other pharmaceutical companies on studies of Luspatercept and other new therapies in EBT in Bangladesh, and funding mechanisms to make Luspatercept available in low income countries

Additional presentations included:

- A video presentation of an electronic patient record being developed by the Ministry of Health for clinical management and tracking of outcomes on a national level.

Action: TIF and international collaborators would be happy to review and make suggestions during development of this electronic clinical record.

- A project developed by All India Facebook, an Indian social and media community to enhance blood donation and blood availability for people living with thalassaemia in Bangladesh. This application has already built a database of blood donors, and has functionality for attracting and registering new donors. Clinics and blood banks can also register and advertise requests for donations. The application also enables advertising of donation camps and donation events. The application is currently active in India, Bangladesh, Pakistan and Brazil and could make a significant contribution to managing blood supply for thalassaemic patients in high prevalence regions of the world.

Recommendation: clinics in Dhaka register with All India Facebook. Also recommend TIF make contact with All India Facebook.

- Prof Anne Dalton. (Genomics, University of Sheffield, UK and Health innovation challenge fund, Department of Health UK) gave a presentation on next generation sequencing and its application to population genetics and health. She emphasized the potential for new technologies in health care provision to provide cross cutting diagnosis and disease stratification through mass screening and mutation analysis. Such technologies could have application for non-communicable disease, addressing the impact of non-communicable disease on population health, economic productivity.

5. Visits To Healthcare Facilities

5.1 Visit to Dhaka Shishu Hospital and Bangladesh Thalassaemia Society Clinic. There has been a significant increase in the number of patients diagnosed and treated at the Dhaka Shishu (Children's) Hospital (DSH) and at the Bangladesh Thalassaemia Society clinic. Data reported in the thalassaemia laboratory of the Children's Hospital show that current registered patients are 3242 of which 2486 (77%) are HbE beta thalassaemia and 756 (23%) beta thalasseamia major. This compares with about 1300 at DSH in 2009.

The Dhaka Shishu Hospital has a DNA laboratory: headed by Professor Waqar Khan and with two scientific officers; M.A Aziz and Sudipa Arka Das. Equipment in the laboratory consists of a PCR Thermal Cycler, real time PCR and a one capillary ABI genetic analyser. Additionally equipment for screening: full blood count, HPLC and Serbia capillary electrophoresis. There is a D10 Biorad Variant which was donated by University College London in 2011

The DNA laboratory has performed 202 prenatal diagnosis for beta thalassaemia major and HbE/beta thalassaemia on chorionic villus samples or amniotic fluid cells. They reported they have had four misdiagnosis using the Amplification Mutation System (ARMS) and have therefore changed their primarily technique to Real Time PCR. Only one women undergoing prenatal diagnosis did not have an affected child reflecting that couples are not offered screening prior to conception. The prenatal diagnosis results showed there were 28 pregnancies with HbE/beta thalassaemia and 12 with beta thalassaemia major and the majority of women terminated their pregnancy. There was no discussion of quality control procedures and if any existed.

5.2 At the Bangladesh Thalassaemia society clinic significant improvements and expansion of the service compared to observations during the visit in 2009. The premises were much improved, processes for transfusion streamlined, and availability of blood has also increased. Transfused units have increased from 7000 in 2014 to nearly 10,000 in 2018, with a commensurate increase in blood donors. Apheresis equipment is also available for provision of platelets.

5.3 DNA Solutions- this is a private DNA laboratory the visit was arranged by father of an affected child, who was in contact with MP prior to the visit, as his wife's prenatal diagnosis at this laboratory was misdiagnosed and his son has HbE/beta thalassaemia. We met with staff at the centre to discuss this case and it was clear that the error occurred due to poor understanding of the genetic tests carried. The laboratory is a modern laboratory with excellent equipment to perform PCR analysis it has separate pre and post PCR areas, there is next generation sequencing equipment which they plan to use for NIPD for screening pregnant women for Trisomy 21, 13 and 18.
We were not given data of the number of prenatal diagnosis carried out in this laboratory and there is no indication of quality control procedures and no indication of an expert in haemoglobinopathies in this laboratory.

5.4 Biomedical Research Foundation

During the visit we met some motivated health scientists from the BRF who presented their work on the lack of knowledge and misperceptions about thalassemia among college students in Bangladesh, this paper has been submitted for publication. Their work so far focuses on:

- College teacher-based thalassemia awareness and prevention: A community-based approach
- Piloting of Blood for Thalassemia (BFT)- A community-based unique blood donation approach by utilizing an underutilized resource to solve blood scarcity in Bangladesh.
- Baseline studies on post-pubertal age groups (college, university and tribal) to understand the knowledge gaps and societal perspectives to prevent thalassemia in Bangladesh.
- Psychological aspects of parents with thalassemia children in Bangladesh.

Collaboration with such groups is essential to disseminate community based information and develop specific research questions e.g. The attitude to prenatal diagnosis and selective termination of pregnancy amongst couples who have children with beta thalassaemia major and HbE/beta thalassaemia.
(**Recommend** international collaboration and training on research variables and validating questionnaires').

6. FURTHER OBSERVATIONS AND DISCUSSION

6.1Clinical care

The commonest syndrome, in the majority of children being transfused in these clinics, is severe Hb E/beta thalassaemia (i.e. phenotypically thalassaemia major), as evidenced by the early age of presentation, growth failure, bone changes, massive splenomegaly, baseline Hb <5. I suspect that the high proportion seen at the severe end of the E thalassaemia spectrum reflects the fact that severe patients are actually seeking medical attention. The milder ones are not diagnosed. In most cases transfusion treatment seemed appropriate.
The clinics have dedicated medical and nursing staff, however, the level of specialist training, especially in the non-hospital clinics, is low, and further training would enhance their ability to decide on appropriate treatment, and to monitor the children's health systematically.
(**Comment**: Training of medical, nursing and laboratory staff remains a high priority in order to implement and national care and prevention programme)
Transfusions for those with thalassaemia major are generally insufficient, and pre-transfusion Hb is generally 6-8 g/dl. Blood for transfusion is either provided by a blood bank associated with the clinic, or blood supplied from one of the other hospitals or blood banks. In some cases, the family bring their own blood which they have obtained and they have arranged compatibility testing at anther other clinic. The blood is screened for HBV, HCV and HIV. In a recent survey of viral infection in 91 multi transfused patients at Dhaka Shishu Hospital, 18.5% were positive for Hepatitis C and 1.1% positive for HbsAg. No HIV positive case was seen.

(**Comment**: Current blood safety data and rates of new infections were not available at the visit. Please could representatives at DSH and BTS clinics provide this data?)
As a consequence of inadequate transfusion, many of the patients with thalassaemia major, have gross splenomegaly, and need splenectomy. Otherwise, they will suffer more from the combined effects of marrow hyperplasia, anaemia, and iron overload. The risks of splenectomy in this population are not known, and reluctance to perform splenectomy is understood.

However, in several cases, the reason for not doing splenectomy was that the families could not afford the pre-splenectomy vaccinations
(One longer term goal may be to undertake collaborative research exploring optimal treatment protocols for BTM and E beta thalassaemia in a low income setting, and collaborating with drug companies (eg Novartis, Apotex, Celgene, Bluebird bio) on how new therapies could be introduced in a low income setting).

(**Comment**: As above, a retrospective study of outcomes of splenectomy in Bangaldeshi patients would be very helpful in determining the short-term and long-term benefits and risks of splenectomy in Bangladeshi patients with BTM and EBT)

All of the patients are inadequately chelated, and serum ferritin levels are well in excess of 2000ug/l. Desferrioxamine is available, but is very expensive, and there are hardly any pumps available. These few are generally used in the clinics to give one or two infusions around the time of the transfusion, rather than taken home. My observation is that desferrioxmine infusions are almost totally impractical in the Bangladesh setting, apart from a tiny minority of very rich

families. Deferiprone (Kelfer) is available in capsule form and is the cheapest chelation drug. I didn't see any cases of severe arthropathy, but suspect that this does occur in this patient population. There have been several cases of agranulocytosis, but I was not informed of any fatal episodes. Deferasirox is available at a lower price compared to Western Europe, and some patients are now using this, but in general, it is not affordable. Deferasirox is also being marketed by the Indian pharmaceutical company Cipla, under a different name. This is probably an infringement of the Novartis patent license. It is unclear how the drug is formulated. There are (unconfirmed) claims that it is not as effective as Exjade at similar dose, and the liver function abnormalities are more common. This product is about half the price of Novartis deferasirox in Bangladesh.

(**Comments**: One longer term goal may be to undertake collaborative research exploring optimal chelation treatment protocols for BTM and E beta thalassaemia in a low income setting, and collaborating with drug companies (eg Novartis, Apotex, Celgene, Bluebird bio) on how new therapies could be introduced in a low income setting.)

Ultrafast T1 weighted cardiac MRI has been piloted for cardiac iron estimation in India and Thailand, and could become a cost effective and convenient means of large scale screening for cardiac iron overload in the future. This could be further developed through collaboration with UK colleagues who are leading on this work (Prof James Moon, Barts Cardiac Centre and Dr Malcolm Walker, UCH)
(Would also make recommendations on severe EBT and beta thalassaemia major: optimal treatment Hb not to fall below 9.5 g and to accompanied by adequate chelation: deferiprone XX mg/kglday, three times a day, or deferasirox XX mg/kg/day. How to monitor ferritin and CMR.)

6.2 Prevention

6.2.1 Screening Policy

There is no prevention programme in Bangladesh. There is no indication that carrier screening is offered at the premarital stage or to pregnant women. The available data for carrier frequency has been collected from relatively small research projects.

It is clear that the development of the thalassaemia prevention programme in Bangladesh is the way forward to reduce the number of affected births. The most feasible option is premarital screening and is the option adopted by most Mediterranean countries and the Middle East and has been implemented in the Punjab region of Pakistan and very successfully in Iran.

In Iran, there is a five level primary healthcare network; medical schools at the highest level, and the rural health houses at the lowest level. Marriage registrars refer prospective couples to the local screening laboratory. The first step is to screen the man. This was chosen to avoid stigmatisation of a carrier woman. If the man is microcytic the woman is tested. When both are microcytic, their Hb A2 level is measured (a value above 3.5% is diagnostic of beta thalassaemia trait). Couples who are at risk i.e. both carry beta thalassaemia and in Bangladesh those where one carries beta thalassaemia are referred to a local designated health centre for genetic counselling about their risk and the options for avoiding their risk – i.e. (a) to separate and find a non-carrier partner, or (b) to marry as planned, and consider the option of prenatal diagnosis to ensure a healthy family. It should be accepted that some couples will decline prenatal diagnosis. As the Primary Health care system in Iran has many similarities to Bangladesh it is recommended that a similar approach is taken.

Pre-marital screening misses already-married at-risk couples who have not yet had a thalassaemic child. Several approaches can be used to detect and inform these couples.

- **Family studies**. Testing can be offered to the relatives of known thalassaemic patients, with the aim of identifying additional at-risk couples within the family. The following points should be noted. (a) Family studies are particularly likely to detect already married at risk couples (and couples considering marriage), where consanguineous marriage is common. (b) The families of carriers detected by screening also contain many carriers and potential at risk couples

- **Antenatal screening**. The option of offering is he policy in England. However the only choice for these couples is either to have prenatal diagnosis and selective termination of an affected pregnancy or to carry on the pregnancy and have the baby tested at birth. This can be considered for married couples.

- **Testing school children**. This is not a successful option of reducing the health burden of thalassaemia. This has been tested in several countries and notably in India where in a large study by Roshan Colah and colleagues, 5682 school children were tested for beta thalassaemia; a follow up study on a cohort 20 years later found that only 25% of them rememberedthat they were a carrier and that no partners were tested before marriage.

6.2.2 Carrier screening

See Annex for carrier screening discussion and algorithms, screening laboratories, DNA Laboratories, technical details, genetic counselling, foetal sampling and prenatal diagnosis

Carrier screening should be provided by designated laboratories with trained staff supported by a network of reference laboratories. Red cell indices, which are the basis of carrier detection, for beta thalassaemia and best measured using automated equipment. This is followed by haemoglobin A2 measurement using automated equipment. This will identify the majority of beta thalassaemia heterozygotes. Serbia capilllary electrophoresis has the advatage of separateding HbA2 from HbE. Automated equipment may be expensive as an investment, but it is more accurate and less labour intensive. Simpler and cheap tests are discussed in the technical annex, but the need to repeat tests, especially in at risk individuals and couples must be considered.

Screening should be followed by expert **genetic counselling** of at risk individuals or couples and this issue is also discussed in the annex. Prenatal diagnosis is offered to all at risk couples and further details are provided in the annex.

7. Summary of recommendations

7.1 For clinical care:

1. National guidelines for clinical management: International delegates and TIF representatives will be happy to review the next version of the guidelines and make further suggestions prior to implementation
2. Clinical guidelines for HbE/beta thalassaemia require more clinical data, such as outcomes, are needed to guide management recommendations. Data collection and clinical research is therefore, advised
3. The level of public awareness. A study was presented by local investigators on this subject: A paper presenting the results of this work have been submitted for publication in a peer

reviewed journal. This and other work of the group will be very helpful in developing a case and for planning strategy for a public awareness strategy and screening programme. The international delegates and TIF would be happy to become further involved in this work.

4. An electronic medical record is being developed: TIF and international collaborators would be happy to review and make suggestions during development of this electronic clinical record.
5. All India Facebook, an Indian social and media community, has developed a tool to enhance blood donation and blood availability. Clinics in Dhaka should register with All India Facebook. Also it is recommended that TIF make contact with All India Facebook.
6. For further research, international collaboration and training on research variables and validating questionnaires, is recommended.
7. Training of medical, nursing and laboratory staff remains a high priority in order to implement and national care and prevention programme
8. Current blood safety data and rates of new infections were not available at the visit. Please could representatives at DSH and BTS clinics provide this data
9. A retrospective study of outcomes of splenectomy in Bangaldeshi patients would be very helpful in determining the short-term and long-term benefits and risks of splenectomy in Bangladeshi patients with BTM and EBT
10. One longer term goal may be to undertake collaborative research exploring optimal chelation treatment protocols for BTM and E beta thalassaemia in a low income setting, and collaborating with drug companies.

7.2 For prevention (see annex for further details and recommendations) :

1. Establishment of a **network of screening laboratories**. There are several options discussed above.
2. Establishment of a **network of DNA laboratories** to carry out prenatal diagnosis. In Dhaka upgrade on the existing laboratory at the Dhaka Shishu Hospital.
3. Establishment of **reference laboratories** for analysis of diagnostic screening and DNA problems. They should be equipped with a DNA gene sequencer and MLPA to identify the rarer mutations which are not identified by the simpler laboratory DNA methods of ARMS-PCR or Real Time PCR. These labs may also test for maternal contamination where the prenatal sample is found to have an identical genotype result to that of the mother.
4. Work towards **laboratory quality control**, and linkage to international quality control systems.
5. Development of a **training programme for genetic counselling** for thalassaemia disorders.
6. Development of fetal sampling **training programme for obstetricians.**
7. Development and **training of scientists and laboratory doctors** in screening, DNA methods and fetal DNA analysis.
8. Thalassaemia **awareness programme** linked to organisations such as the BRF.
9. **Systematic collection and analysis of**:
   Screening and Prenatal diagnosis data
   Epidemiological questions: studies undertaken for different purposes may be compared and contrasted to obtain greater depth of information about the impact of thalassaemia disorders on families, and attitudes to carrier testing and prevention. For example, data on the uptake of prenatal diagnosis and acceptance of termination of pregnancy and data on how many at risk couples still proceed to marry after screening.
   Training on data collection
10. A national registry for affected births
11. **National Prevention Committee** to include senior scientists, haematologists, obstetricians, genetic counsellors

8. ACKNOWLEDGEMENTS

- Mr Omar Rabbany and the wonderful staff, volunteers and patients at Bangladesh Samity Hospital

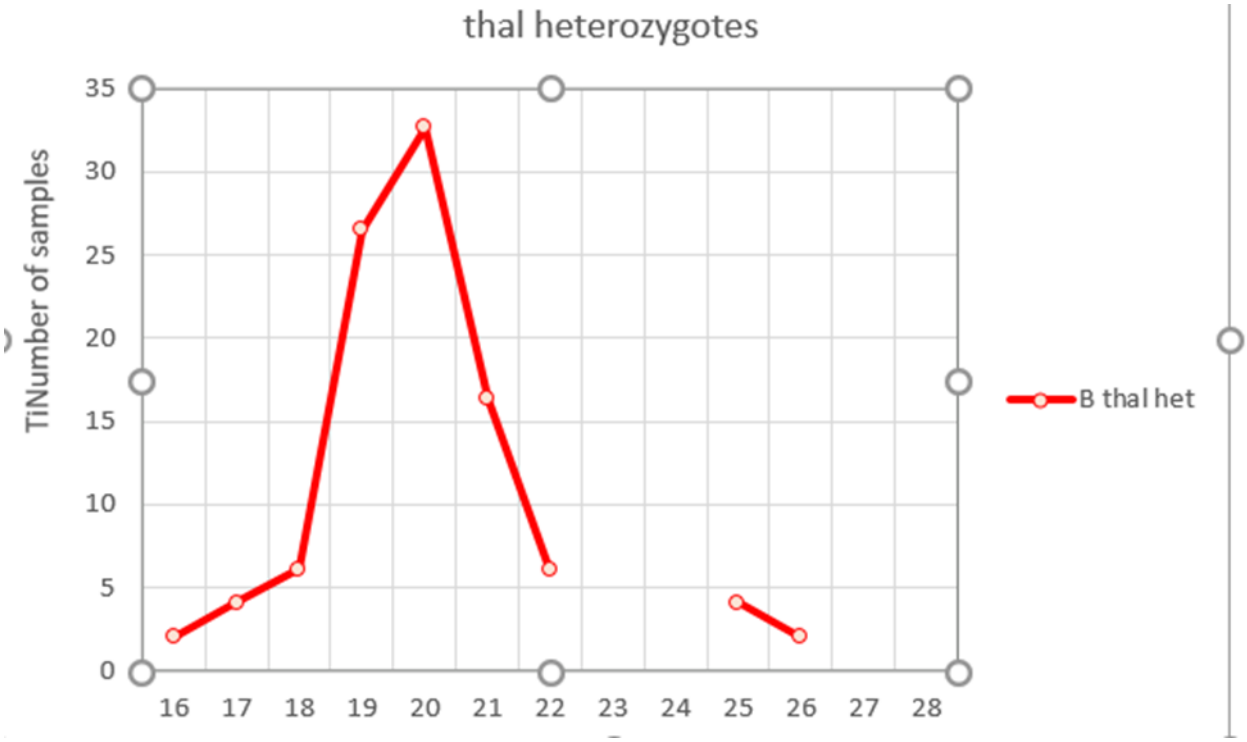
- Youth Club of Bangladesh for their dedication and support during the visit
- Directorate General of Health Services, Ministry of Health
- Staff at Dhaka Shishu Hospital
- UCL for providing some financial support

Annex: Carrier screening, genetic counselling, foetal sampling and prenatal diagnosis– technical details

Carrier screening –beta thalassaemia

Carrier screening should be provided by designated laboratories with trained staff supported by a network of reference laboratories. Red cell indices are measured using automated equipment. When MCH < 27pg then haemoglobin A2 is measured using HPLC or [1]Serbia capillary electrophoresis. This will identify the majority of beta thalassaemia heterozygotes as shown in figure 1. The data is from a small study at the Bangladesh Thalassaemia Samity Hospital showing that the majority of beta thalassaemia carriers have an MCH below 22pg. However there are a few carriers with an MCH between 25-26pg. For more accurate data a larger data set will need to be analysed.

Fig 1. MCH data from beta thalassaemia heterozygotes from Bangladesh Thalassaemia Samity Hospital

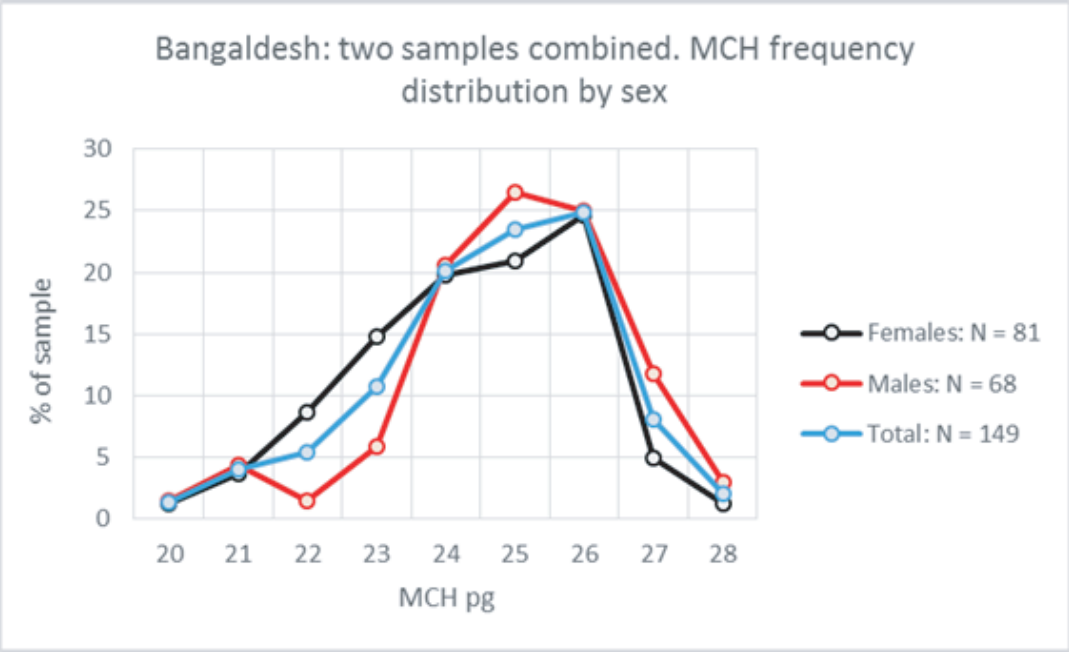


[1] Serbia capillary electrophoresis has the advantage over HPLC as it separated HbA2 from HbE

Carrier Screening- HbE

It has been suggested that HbE carriers are tested by Serbia capillary electrophoresis/HPLC if they have an MCH less than 27pg. To test if this algorithm would miss HbE carriers, screening data from two centres in [2]Bangladesh was compared with screening data from two centres in [3]London. Figures 2, 3 and 4 show the MCH size distribution of HbE carriers in Bangladesh and London.

Fig 2. Bangladesh HbE heterozygotes: MCH size distribution by sex



Males give a symmetrical curve: genetically determined frequency distribution. Females skew to the left representing iron deficiency.

[2] Kindly provided by Mr Omar Rabbany from the Thalassaemia Samity Hospital and Prof Waqar Khan from Dhaka Shishu Hospital

[3] Data provided by Dr Petrou at University College Hospital and Dr Telfer at the Royal London Hospital

Fig 3. **Bangladesh *HbE heterozygotes: MCH size distribution*** ak

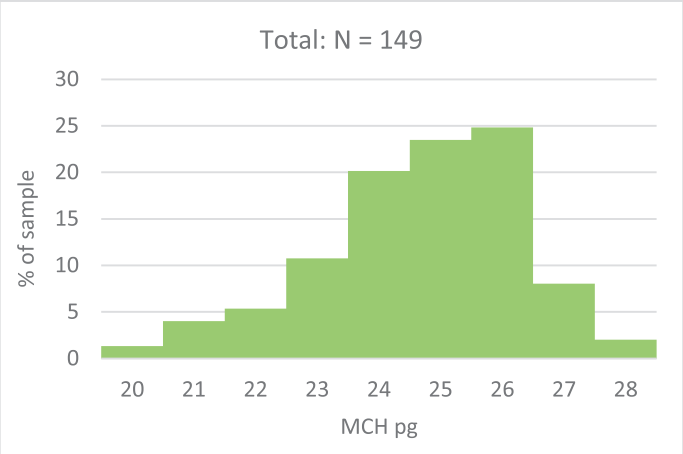
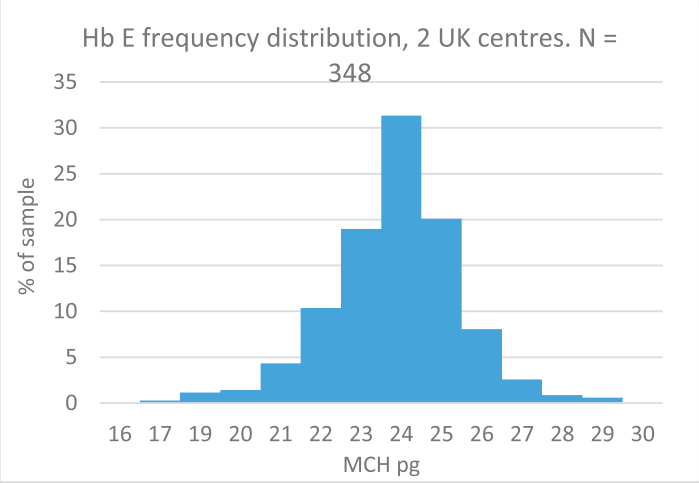


Fig 4. London HbE heterozygotes: MCH size distribution



The Bangladesh data shows, that if MCH< 27pg is taken as the cut off for screening for HbE carriers then only 94% of females and 85% of males will be identified. 90% of samples have an MCH <27pg. With this algorithm 6% of females and 15% of males will be missed by screening.

However the London data shows that 96% on carriers have an MCH< 27pg. This may represent a difference in laboratory standardisation.

Screening large populations requires a financial commitment and political will. There are several screening approaches that may be considered in a premarital screening programme. The Ministry of Health in Bangladesh will need to appraise the different screening options to determine which is the most suitable and acceptable to the population.

Here we discus two possible screening approaches.

Screening Approaches that may be considered:

Approach 1: Screen the male partner first for beta thalassaemia and HbE and screen the female only if the male partner is a carrier

Screen the male partner for beta thalassaemia and HbE by FBC and Serbia capillary electrophoresis/HPLC

If the male has HbAE, the only genetic risk is HbE/beta thalasaemia. Therefore screen the female **only** for beta thalassaemia **by FBC** and only measure the HbA2 if the MCH<27pg.

If the male partner has beta thalassaemia trait the genetic risk is beta thalassaema major or HbE/beta thalassaemia. Therefore screen the female partner for beta thalassaemia and HbE by FBC and Serbia capillary electrophoresis/HPLC

This policy will not detect all HbEE.

Based on this algorithm Table 1 estimates the number of tests required annually in both males and females in each division in Bangladesh.

[4]The estimates are based on the age distribution of the population, that one fifth of the population is aged 20-24 and that they all marry. Therefore:

- 235287 males and 47600 females will require testing for beta thalasaemia and HbE,
- 187686 females will require testing for beta thalassaemia only

It is important to note that if a cut off MCH<27pg is used for carrier screening for beta thalassaemia then approximately [5]50% of individuals will have an MCH <27pg due to beta thalassaemia, alpha thalassaemia and iron deficiency and will require confirmatory testing by Serbia capillary electrophoresis/HPLC. If the MCH cut off is reduced to <25pg, this will reduce the number of confirmatory tests required, but will miss carriers of beta thalasaemia.

[4] Note: Annual new couples' estimates: in Bangladesh males aged 20-24 years is 4.8% of the total population. If all males marry, annual marriages are equivalent to 4.8% of the population, (population in 2017 was 164,827,717 divided by 5. However, the number of males marrying will actually be less, as not all will marry.

[5] Data in this report from papers prepared for publication: Modell, Petrou et al, General Method for establishing the Global Epidemiology of Congenital Disorders; Haemoglobin Disorders.
Modell B, Darlison M, Petrou M, A General Method for establishing the Global Epidemiology of Congenital Disorders, Haemoglobin disorders, G6PD deficiency and normal red cell values

Table 1. Estimates for the carrier tests for males and females using Approach 1

	Tests for Males only		% β thal Heterozygotes and HbE				Annual carriers detected(males only tested)				Annual partner tests (females only tested)	
Division	Annual new couples (1% of pop)	Annual carrier screening tests	Het β thal	HbAE	HbEE	Total healthy carriers	Het β thal	HbAE	HbEE	Total	Het β thal and HbE tests (i.e.males have βthal)	β thal test only (i.e the male is HbAE or HbEE),
Rangpur	157,878	157,878	3.4	30.4	3.8	37.5	5,324	47,916	5,947	59,187	5,324	53,863
Rajshahi	184,849	184,849	3.8	11.9	0.4	16.1	6,981	21,939	793	29,712	6,981	22,732
Khulna	156,878	156,878	1.2	4.9	0.1	6.1	1,906	7,624	102	9,632	1,906	7,726
Mymensingh	113,700	113,700	2.4	9.3	0.2	11.9	2,687	10,523	282	13,492	2,687	10,805
Dhaka	360,544	360,544	2.4	9.3	0.2	11.9	8,520	33,369	894	42,783	8,520	34,263
Barisal	83,257	83,257	2.4	9.7	0.3	12.4	2,017	8,067	227	10,311	2,017	8,294
Sylhet	99,102	99,102	6.6	6.6	0.1	13.4	6,555	6,555	135	13,246	6,555	6,691
*Chittagong	284,230	284,230	4.8	14.1	1.2	20.0	13,611	39,992	3,320	56,923	13,611	43,312
TOTAL	1,440,437	1,440,437	3.3	12.2	0.8	16.3	47,600	175,986	11,700	235,287	47,600	187,686

Approach 2: Screen the male and female by a full blood count (FBC).

An alternative screening algorithm that may be considered;

Screen the male and female by FBC.

If one or both have an MCH <27pg, then test both by capillary electrophoresis/HPLC.

This algorithm will identify at risk couples for HbE/beta thalassaemia and Beta thalassaemia major. This algorithm and will reduce the number of capillary electrophoreses/HPLC tests carried out.

Simpler methods for carrier screening in rural settings:

- Naked Eye Single Tube osmotic Fragility (NESROFT): This is a simple method used for screening for beta thalassaemia based on the osmotic fragility and is still used in some low resource countries to screen large rural or tribal populations. This test will also identify microcytosis due to iron deficiency and microcytosis due to HbE. **Recommend a pilot study using NESTROFT and capillary electrophoresis to validate.**
- DCIP (Dichlorophenol Iodophenol Precipitation): Methods for screening for HbE; In Thailand DCIP (Dichlorophenol Iodophenol Precipitation) has been used successfully for screening in the rural districts. The use of this test would reduce the number of samples requiring referral to a central laboratory. There are disadvantages to this test as sometimes it can be difficult to read. **Recommend a pilot study using DCIP and Capillary electrophoresis to validate.**
- Immunoassay test is being developed by Silver Lake Research corporation, for HbE similar to HemoTypeSC (www.hemotype.com) test. The test will be able to combine simultaneous detection of HbA, HbS, HbE, and HbD, and also having standalone tests for HbA/HbE. This assay will have the potential to be offered in the rural areas. The cost per test would be between $1.00 to $2.00.

Carrier screening laboratories

Laboratory staff should be aware that the programme stands or falls on the quality of carrier diagnosis. There will be concerns because of unresolved problems that could lead to carrier misdiagnosis as there will be:

- The unknown prevalence of unusual forms of thalassaemia (normal Hb A2 beta thalassaemia trait, truly silent beta thalassaemia trait, alpha thalassaemia trait etc), and how to interpret borderline HbA2 values. A common thalassaemia mutation found in the South Asian population is Cap +1 (A>C) (HGVS:HBB:c.-50A>C) often presents with borderline or normal HbA2 values.

- How to interpret microcytosis with a normal HbA2 when iron deficiency has been excluded (a common problem in many thalassaemia screening programmes). Most of these will be due to harmless alpha-plus thalassaemia.

- How to interpret atypical MCH data.

A haematology reference centre where the unusual haemoglobins are investigated further using techniques such as isoelectric focusing, Serbia Capillary electrophoresis, HPLC for separating variant haemoglobins would be required. This can be linked to the reference DNA laboratory.

Laboratory quality control:

- Laboratory screening techniques should be evaluated. International reference materials for HbA2 should be used.

- Laboratories should work towards ISO 15189 an international standard for a quality management system for medical laboratories

- Consider joining an external quality assessment scheme, such as UKNEQAS. This scheme is open to overseas laboratories and runs several schemes such as blood counts, HbA2, abnormal haemoglobins. UK NEQAS Haematology on behalf of the World Health Organisation also organises an International Scheme (IEQAS Haematology) which has been designed mainly for developing countries. Contact details UK NEQAS, PO Box 14, Watford, WD18 OFJ. fax: 0044 1923 217879 e-mail: haem@ukneqas.org.uk

Collection and rigorous analysis of existing screening data:

Persisting uncertainties about the accuracy of screening and definitive diagnosis of at risk couples will be resolved only when good data is available on the following:

- Prevalence of carriers of atypical forms of beta thalassaemia, including normal Hb A2 beta thalassaemia and truly silent beta thalassaemia, by district.

- Prevalence and types of microcytosis, by district

- Response of microcytic individuals to iron therapy, by sex, age and district.

Baseline data is needed against which to assess results of thalassaemia screening. This can be obtained by collecting unselected data on a minimum of 1000 sequential screening tests for males and 1000 for females in each district. This should include results of partner tests, and

response to iron therapy when this is given. International delegates and **TIF representatives will be happy to help to analyse this data and make practical recommendations.**

DNA Laboratories

1. DNA Laboratory network.

The network of DNA labs would be required where prenatal diagnosis samples can be analysed. Initially to include one reference laboratory in Dhaka, and subsequently DNA laboratories in the other Administrative Divisions. The laboratory at the Dhaka Shishu Hospital should be upgraded with the provision of improved equipment. At risk couples who proceed to marry should be referred to these laboratories for preliminary genetic work-up and genetic counselling and the offer of prenatal diagnosis. **The Ministry should establish a National register of Prenatal Diagnosis**

2. Review of literature for thalassaemia mutations present in Bangladesh

Prior to the visit Dr Mary Petrou carried out a literature review on the thalassaemia mutations in Bangladesh. Table 2. Shows data adapted from three published papers. The frequency data was adjusted to remove the HbE when included in the study. The data shows that 63-74% of beta thalassaemia carriers have the common IVS 1-5 (G>C) ([HGVS-HBB: c.92+5G>C] beta thalassaemia mutation. If the most common five thalassaemia mutations in Table 2 are tested, then 60-95% of the mutations will be identified.

To consider the most cost effective methods of DNA analysis a way forward for consideration is for that the local DNA laboratories to screen either for the five most common mutations using ARMS-PCR, or only screen only for the most common IVS 1-5 (G>C) thalassaemia mutation. If no result is obtained, the DNA can be sent to the reference DNA laboratory for further testing. The local DNA labs can then proceed with prenatal diagnosis samples using either real-time PCR or ARMS-PCR for the common mutations that are assigned to their laboratory. This protocol will reduce the number of expensive equipment such as gene sequencing required in each laboratory.

It was also clear from papers reviewed that there is a training requirement for scientists/doctors in DNA laboratories, as a number SNP's of no clinical significance were assigned as pathogenic mutations and a haemoglobin variant mutation assigned as a beta thalassaemia mutation. **A training programme for laboratory personnel is recommended and linked to international laboratories with the help of TIF.**

Table 2. Mutations present in Bangladesh- review of literature

Mutations in Bangladesh	Frequency % (Banu et al 2018 Beta thalassaemia carriers :parents of affected patients in Dhaka)	Frequency % (Chaterjee et al 2015 Beta thal major and HbE/B thal in Chittagong)	% only Beta thal	Frequency % (Aziz et al 2017. 100 Beta thal major and HbE/B thal in Dhaka)	% only beta thalassaemia
IVS 1-5 (G>C)	63.00	39.1	67	55.5	74
Codon 30 (G>C)	18.00	3.5	6.0	1.0	1.0
Fr 8/9 (+G)	5.0	2.7	5.0	3.5	5.0
Fr 41-42 (-TTCT)	4.0	4.3	7.0	3.0	4.0
Codon 16 (-C)	3.0				
-90 (C>T)	2.0	1.9	3.0		
IVS1-130 (G>C)	2.0				
IVS 1-1 (G>T)				1.5	2.0
Codon 15 (-T)	1.0				
-29 (A>G)	1.0				
Codon 15 (G>A)		1.9	3.0		
Codon 30 (G>A)		1.6	3.0		
Codon 15 (-T)		1.6	2.0		
IVS1-130 (G>C)		1.6	2.0		
619bp deletion		0.8	1.0		
Codon 16 (-C)		0.8	1.0		
Codon 26 (GAG>AAG) HbE	Not studied	40.2	Subtracted	25	Subtracted
Percentage Unknown Top 5 mutations	0 95%		0 88%	10.2	14 60%

3. Quality Control

- All the DNA laboratories in the programme should join an external quality assessment scheme, such as the UK NEQAS DNA haemoglobinpathies scheme. Contact haem@ukneqas.org.uk address as above.

- National standards for DNA diagnosis of the haemoglobinopathies should be produced, based on the international DNA guidelines.

- Establish a DNA laboratory network with meetings at regular intervals to discuss diagnostic problems and service developments.

4. Future studies on “obligate carriers” of beta thalassaemia (parents of beta thalassaemia major children)

- Molecular analysis for beta globin mutations should be performed on a selected sample, especially when parents have atypical or normal red cell indices.

- A random sample of patients and obligate carriers to be studied for alpha globin gene mutations. The families are essentially unselected with regard to alpha thalassaemia, and so provide a convenient random sample of individuals whose DNA is already available.

We would happily help to analyse this data, and make further practical recommendations on the basis of the findings.

These recommendations made for DNA laboratories are international standards. It is however recognised that prenatal diagnosis can be offered at a lower cost using different but acceptable standards in a low resource setting. For example, Prof Suhaib Ahmed in Pakistan has developed a laboratory which tests over 1800 prenatal samples per year. He offers testing at a fraction of the expected cost. **Recommend collaboration with Prof Ahmed**

Genetic counselling

1. Communication skills.

A training programme should be set up to train health professionals in genetic counselling. This should be complimented by the development of patient information materials such as those freely available on the web http://2000.apogi.info/ APoGI (Accessible Publishing of Genetic Information)

Genetic counselling requires particular skills of communication. The responsibility in genetic counselling should not be underestimated. Misinformation can have disastrous consequences Couples should be allowed to make their own informed decisions and should not be directive to discourage carrier couples from marrying if that is their choice. Continuing emphasis needs to be placed on the fact that ethical genetic counselling is “non-directive” - statements such as “convince couples not to marry” should not be used.

2. Parental consanguinity

It is often perceived that consanguinity is the cause of genetic disease. This was also the opinion of several delegates at the conference. It should be borne in mind that the majority of consanguineous couples do not have children with thalassaemia. A couple who are at risk of thalassaemia regardless of whether they are related or not, has the same genetic risk as having an affected child. Marrying a close relative increases the chances of marrying another carrier and increases the prevalence of affected patients in the population. It is, however, counterproductive to aim to disrupt such traditions which are deep rooted in society, instead it is much better to offer screening for genetic diseases and provide accurate information to couples.

Foetal Sampling

Obstetricians should be trained to collect samples by chorionic villus sampling (CVS) rather than by amniocentesis. CVS can be performed from 11 weeks gestation and in the second and third trimester of pregnancy. However, it should be encouraged to be offered early in pregnancy so that if a termination of pregnancy is requested due to an affected foetus, then it can be performed in early pregnancy which is more acceptable to women. Additionally, most Muslim countries have a religious decree allowing termination of pregnancy only up to 120 days of foetal life (17 weeks). **A training programme should be provided for obstetricians and this can be complimented by arranging workshops with international experts.** Such a workshop was arranged by TIF at the Dhaka Shishu Hospital in 2010.

Pilot studies to access the feasibility of premarital screening in Dhaka

To evaluate the feasibility and the acceptance of a prevention programme in Bangladesh recommend a pilot study of premarital screening in a district of Dhaka such as Dhaka District. Collection of screening data and information on choices ‘at risk’ couples make i.e. do couples separate and find a non-carrier partner or marry as planned and consider the option of prenatal diagnosis? **The international delegates and TIF would be happy to advise and to review data.**

The DNA laboratory at Dhaka Shishu Hospital can be included in the study.