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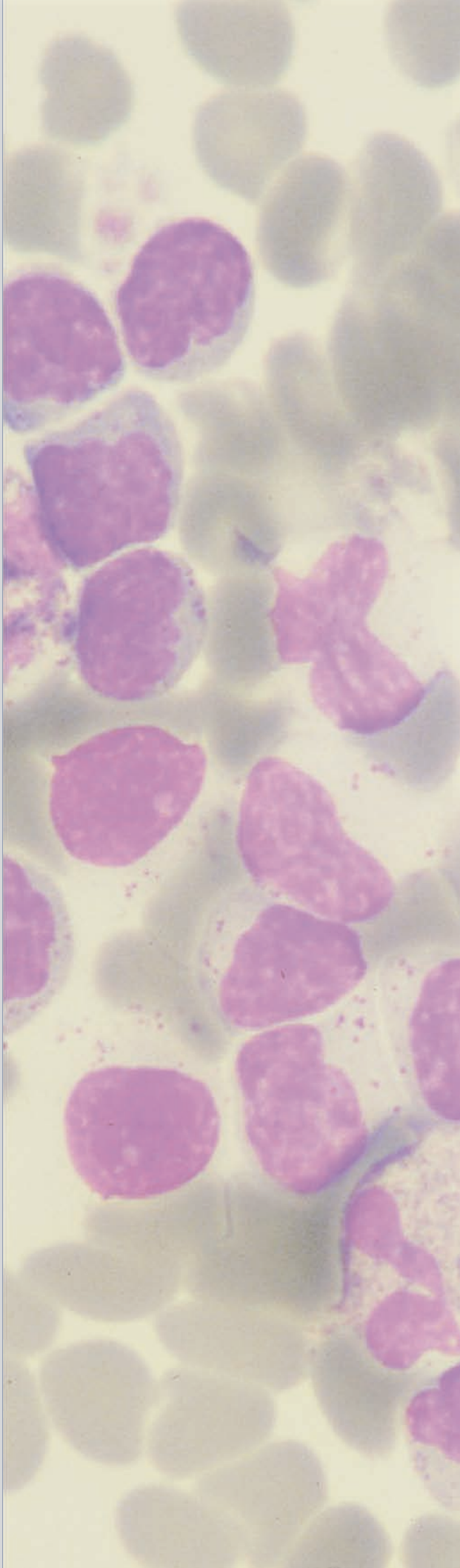
1st Pan-Asian Conference on Haemoglobinopathies

8-10 February 2012, Bangkok – Thailand

THALASSEMIA REPORTS

ISSN 2039-4357 - eISSN 2039-4365

pagepress



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THALASSEMIA REPORTS

is published by PAGEPress Publications, Italy.
The journal is completely free online at www.thalassemiareports.org
Publishing costs are offset by a publication fee charged to authors.

For more information
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Correspondence

Our publishing offices are located
in via Giuseppe Belli 7, 27100 Pavia, Italy.
Our telephone number is +39.0382.1751762
and our fax number is +39.0382.1750481.
E-mail: info@pagepress.org

1st Pan-Asian Conference on Haemoglobinopathies

8-10 February 2012
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ORGANISED BY

Thalassaemia International Federation (TIF)

IN COLLABORATION WITH THE
Thalassaemia Foundation of Thailand (TFT)

AND

Mahidol University (MU)

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8-10 February 2012, Bangkok - Thailand

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EMRO

CURRENT SITUATION, CONTROL STRATEGIES AND HEALTH SYSTEMS IN ASIA, PAKISTAN

Jovaria Mannan

Professor of Paediatrics and office bearer of Pakistan Paediatric Association, Heal Trust Pakistan and The Eisaar Trust Pakistan

Pakistan is a very diverse country with 6 provinces and a total population of approximately 180 million. The health delivery in Pakistan is primarily based in the private sector with more than 70% care in the private sector on a fee for service basis. Only 30% is catered for by the public sector and in a rough estimate an individual visits a Primary care center only once a year. The Healthcare infrastructure is significantly lacking, with the current population statistics we have only one third of the required number of doctors available. The healthcare system in the public sector is based on providing healthcare at a Primary level by Basic Health Units (BHU's) and Rural Health Centers whereas a majority of our population utilizes Traditional Medicine, Faith healers, Hakims and Homeopaths. Keeping this in mind the healthcare provision for thalassaemia patients tends to be very haphazard in the public sector which is based on diagnostic, critical and transfusional services. Non-Governmental Organizations and Welfare Societies care for a majority of patients providing preventative, awareness related and transfusional care. Three of the six provinces currently have a prevention programme approved and to some extent initiated. Though Thalassaemia federation of Pakistan has done a lot of awareness without the political will and financial support of the government an effective treatment and prevention programme cannot succeed.

SEARO

THALASSAEMIA IN BANGLADESH

Ahmed Waqar Khan

Dhaka Shishu Hospital Thalassaemia Centre, Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh

Objective. Thalassaemia is the most common congenital disorder in Bangladesh. There is no national registry for documentation of the number of thalassaemic patients born and no data regarding the number of thalassaemic in the country. There is no government policy for prevention of thalassaemia. Public awareness of the disease is very low and knowledge of thalassaemia is poor. Thalassaemia intermedia are unduly treated with regular transfusion as knowledge of thalassaemia intermedia is very poor. This paper gives the situation of thalassaemia in Bangladesh
Methods. Collection of data of situation of thalassaemia in Bangladesh is presented. Most of the data is from Dhaka Shishu (Children) Thalassaemia Centre, the largest centre for diagnosis, care and management of thalassaemia.
Results. Hb E beta thalassaemia is common and nearly 85% of cases are Hb E beta thalassaemia. Alpha thalassaemia has not been reported. Carrier status of Hb E is 6.1% and beta thalassaemia trait is 4.1%.with wide variation in different regions of Bangladesh and expected births of thalassaemic children is about 6000 to 7000 per year. HCV positivity was seen in 18.5% and 13.8% and HbSAg positivity in 1.1% and 12.5%.in multi-transfused patients in two different centres. The most common mutation seen was IVS 1-5(G-C). Drugs are not widely available other than in big cities. Blood is somehow managed but recommended Hb level is not achieved in majority of patients. Pre natal diagnosis is not as yet fully established.
Conclusions. Thalassaemia is a significant health problem and efforts are needed both by the Government and non government associations needed for its prevention and adequate treatment.

CONTROL STRATEGIES FOR HEMOGLOBINOPATHIES IN INDIA

Roshan Colah

Scientist F (Deputy Director [SG]) National Institute of Immunohaematology(ICMR), KEM Hospital Campus, Mumbai
Email: colahrb@gmail.com

Population statistics. India is the second most populous country in the world where 17.3% of the global population (1.21 billion people) reside according to the Census of India-2011. The population comprises of several castes and tribal groups. 8% of the population is tribal individuals. Many of these tribal groups have remained relatively isolated from the mainstream of society. The average literacy rate is 74% and it varies from 64 to 94% in different states. 50% of the population is young being less than 25 years of age. There is great ethnic, cultural, religious and genetic diversity in this vast country.
Extent of the problem. The β-thalassaemias and sickle cell disorders are a major health burden in India and they lead to considerable morbidity and mortality. The first case of thalassemia in the country was recorded in a 2 ½ year old Bengali boy in 1938. Epidemiological studies done over 4 decades in different regions of the country have shown that the prevalence of β-thalassemia carriers varies from 1-17% in different ethnic groups with the overall rate of

carriers in the country being 3-4%. Thus, there would be at least 35-45 million carriers and it is estimated that 10,000 to 12,000 babies with β-thalassemia major would be born each year. Recent micro-mapping studies in western India have shown that there is considerable variation in frequencies of carriers of β-thalassemia even within each state. Such studies are important to determine the true burden of the disease. It is estimated that there would be around 100,000 β-thalassemia patients in the country. Majority of these children do not receive optimum treatment with regular blood transfusions and adequate iron chelation. Bone marrow transplantation is available at few centres but is not affordable by majority of families. There is no national survival data available as yet. Lehman and Cutbush first reported the presence of the sickle gene among tribal communities from the Nilgiri Hills in South India in 1952. Subsequent surveys showed that sickle cell anemia was a major problem in central India and the tribal belts in the west, east and south especially in the states of Madhya Pradesh, Chattisgarh, South Gujarat, Maharashtra and Orissa. Although it is mainly seen among tribal population groups it is also present in some non-tribal communities. The prevalence of sickle cell carriers is as high as 30-40% in some of these population groups. Estimates indicate that more than 5000 babies with sickle cell anemia would be born each year. Hb E is present mainly in eastern and north eastern India and the presence of Hb E carrier ranges from 1-10% in West Bengal and is as high as 20-64% in some population groups in Assam, Meghalaya and Tripura in the north eastern region. Hb E-β-thalassemia is the commonest hemoglobin disorder in West Bengal.

α+thalassemia [(-α/αα) and (-α/-α)] is prevalent both in the caste groups (3-23%) as well as tribal groups (17-97%) and α gene triplication has also been reported in 1-2% of individuals.

Awareness in the population. A major constraint in India is reaching out to a population of over a billion people where an overwhelming majority (>70%) live in rural areas in around 600,000 villages. The only way to reach the grass roots is to utilize the existing public health infrastructure and involve the local community leaders and health care workers. A multilingual portal on rural healthcare provides useful health care related information but it should also incorporate information on genetic disorders like the haemoglobinopathies. Multi-centre studies done by the Indian Council of Medical Research (ICMR) have highlighted that awareness on the hemoglobin disorders is lacking even in urban areas in different states (7 to 50% among college students and 0.2 to 20% among antenatal women). In a study reported from Gujarat, awareness about the thalassaemias was seen in only 19% of MBBS doctors. Late registration at antenatal clinics, husbands often not being available for screening and social stigmatization are other problems which will have to be addressed.

Technology used in screening programmes. Screening has been done in schools, colleges and antenatal clinics, eitherby individual institutions or as multi-centre studies as well as among extended family members of affected children. The latter was found to be the most cost effective approach. Most of the screening programmes now use red cell indices and HPLC analysis for quantitation of Hb A_2 levels. However, in resource poor settings and when facilities are limited in remote areas, the naked eye single tube osmotic fragility test is being used. Borderline HbA_2 levels are increasingly being encountered and need careful evaluation for accurate identification of couples at risk of having a severely affected child. These have often been associated with β thalassemia mutations like the capsite+1(A>C), -88(C>T) and polyA (T>C) but have also been seen sometimes with common mutations like IVS1-5(G>C), codon 15(G>A) and codon 30(G>G) and in a few β thalassemia heterozygotes with associated β-thalassemia or α gene triplication. The National Rural Health Mission has started screening for haemoglobinopathies in a few states. Gujarat is the first state in India to incorporate a Sickle Cell Anemia Control Programme in the existing government health services of the state. In South Gujarat where sickle cell disease is prevalent, 78 centres including 44 Primary Health Centres (PHCs), 29 Community Health Centres (CHCs), 3 District General (Civil) Hospitals, 1 Government Medical College (GMC) and one NGO are all equipped with primary screening facilities. Awareness and education programmes are conducted in the local language and medical officers, laboratory technicians and counselors are given training regularly. In West Bengal, screening and counseling programmes for β thalassemia and HbE-β thalassemia have been started in different districts.

Strategies for control of haemoglobinopathies. It is well accepted that a nationwide programme for effective control of these disorders is needed. However, there is no National Control Programme for the hemoglobin disorders as yet but there are around 10-12 centres in academic Institutions and large hospitals where prevention programmes are being undertaken since several years. Facilities for both first trimester prenatal diagnosis by CVS and DNA analysis and second trimester diagnosis by cordocentesis and fetal blood analysis are available. Extensive studies on characterization of β thalassemia mutations have been done in over 10,000 β thalassemia alleles using ARMS, reverse dot blot hybridization and DNA sequencing. More than 65 mutations have been characterized in the Indian population and 7 mutations have been shown to account for 85 to 90% of the molecular defects. Their regional distribution is now known and certain rare mutations have been commonly found in some communities (*e.g.* Codon 5(-CT) among the Prajapatis in Gujarat and Codon 110 (T>C) among the Agri community in Maharashtra). Our experience in doing prenatal diagnosis in around 2500 pregnancies has shown that it is acceptable by all the communities including tribal groups although majority of the couples still come retrospectively after having an affected child.

The feasibility of a control programme for sickle cell disease has been shown by a field based study done in the Wyanad district in Kerala where mass screening in tribal communities followed by marriage counseling and prenatal diagnosis was possible. Our studies in and around Nagpur in central India have also shown that many couples at risk of having a child with sickle cell disease opt for prenatal diagnosis. The Indian Council of Medical Research (ICMR) has now taken the lead to start a National Haemoglobinopapthy Control Programme

which will include both the β-thalassaemias and sickle cell disorders. As it is not feasible and financially viable to start the programme simultaneously in all states, it will be done in phases and will soon begin in Delhi, Chandigarh and Punjab in north India as a pilot study as infrastructure and expertise is available at several centres in this region. Eventually the programme would be integrated in the existing health care system involving the Central and State Governments as well as NGOs. Training of staff and quality assurance programmes will be an integral component of the programme both for screening and molecular diagnosis. An initiative has also been taken by ICMR to establish 5 additional regional centres mainly in medical colleges in Maharashtra, Gujarat, West Bengal, Karnataka and Punjab for molecular and prenatal diagnosis of β-thalassemia and sickle cell disorders. These centres will soon start functioning independently. Besides this, hands - on workshops are being organized regionally in medical colleges under one of the translational research programmes of ICMR where National Institute of Immunohaematology has already given training in screening and molecular diagnosis of haemoglobinopathies to pathologists, hematologists and laboratory technologists from 34 medical colleges from 4 states. It is hoped that this exposure will empower them to get involved in prevention programmes in their regions. Newborn screening for sickle cell disorders has been initiated since the last 2 years in Gujarat, Chattisgarh and Maharashtra both in tribal and non- tribal groups with a high prevalence of Hb S as early intervention can avoid complications and comprehensive care would reduce morbidity and mortality. A cohort is being raised and these babies are followed -up regularly. Preliminary studies have shown that sickle homozygous babies in the non- tribal group in Maharashtra have a more morbid presentation compared to tribals from Gujarat. This programme has also resulted in greater awareness among the parents of sickle homozygous babies who are opting for prenatal diagnosis in subsequent pregnancies.

CURRENT SITUATION OF THALASSEMIA CONTROL STRATEGIES IN INDONESIA

Pustika Amalia Wahidiyat

Thalassaemia Centre, Division of Hematology-Oncology, Department of Child Health, Cipto Mangunkusumo Hospital Jakarta, Indonesia

Background. As a genetic disorder thalassemia is still an incurable disease until this time, but can be prevented by premarital screening and prenatal diagnosis (PND). Gene frequency in Indonesia is quite high, β-thalassemia 3-10%, α-thalassemia 2.6-11% and HbE 1.5-36%. There are at least 5,000 thalassemia patients registered from all over Indonesia, but this number is far below the real number found in population. The economic burden of lifelong blood transfusion and iron chelation therapy can be reduced by a national thalassemia prevention program.
Objective. To describe prevention strategies of thalassemia in Indonesia.
Methods. Data were obtained from reports of awareness and education programs, prevention pilot studies on premarital screening during 2009-2011 and PND from Eijkman Institute for Molecular Biology Jakarta during 2000-2011. Results from education and prevention programs were presented to the Ministry of Health.
Results. Socialization through seminars, counseling and prospective screening to increase public awareness on thalassemia started in 2009 and has reached several cities in Indonesia. The participants from various background from layman to general practitioners and physicians. Prospective screening resulted in 5.9% (19 out of 324) β-thalassemia trait and HbE trait. Screening among 420 medical students University of Indonesia revealed the prevalence of β-thalassemia trait and HbE trait were 4.76%. Antenatal screening and PND started in 1998, total PND was 84 cases. The results were 20% compound heterozygotes or homozygote, all of them except 1 affected fetus were terminated. Two were misdiagnosed, both were β-thalassemia compound heterozygotes, but prenatally diagnosed as heterozygote. In June 2011, the Ministry of Health launches Health Technology Assessment on thalassemia screening.
Conclusions. Thalassemia prevention program in Indonesia has been done as pilot projects starting 1998. It appears that there are many obstacles to bring this issue as a national program, resulting in escalating number of new cases every year. Designing and starting the optimal management and prevention in Indonesia is not easy because of demographic, ethnicity, and diversity of mutations and phenotypes. The most important factor is government's commitment for prevention program as this will improve overall morbidity and reduce treatment cost.

AN OVER VIEW OF NATIONAL THALASSAEMIA PROGRAME IN MALDIVES

Farzana Khatoon

Thalassaemia Centre in M'ale, Maldives

Maldives consists of a group of small islands with a total population of 330,000. Total number of Islands in Maldives: 1192. Number of Inhabited Islands are200, Number of Uninhabited Islands are 992. Thalassaemia is a huge Public Health concern in Maldives. Beta Thalassaemia Carrier rate in Maldives is about 18% to 20%. Total number of Beta thalassaemia patients and patients with other haemoglobinopathies registered in Maldives till April 2011 726. Number of patients taking treatment regularly at National Thalassaemia Centre are 292, and in other islands 253. Under the National Thalassaemia Program, National Thalassaemia Centre was established on 29th December 1994. It is a day care centre and its working hours are from 0800 – 2000Hrs. National Thalassaemia program have two components:
A) Curative
B) Preventive
Curative treatment consists of:

1) Conservative treatment
- Blood transfusion

Blood transfusion service is provided at

a) National Thalassaemia Centre - Male'
b) Indira Gandi Memorial Hospital - Male'
c) 6 Regional Hospitals

d) 69 Atoll Hospitals and Health Centres
- Iron chelation

2) Permanent – Bone Marrow Transplant (BMT).
- So far 32 patients have undergone BMT at different centres in countries such as Italy, India and Thailand.

Preventive program consists of:
1) Population screening for Thalassaemia
2) Genetic Counseling
3) Creating awareness by Mass Media
4) Advice for Prenatal Diagnosis – facilities not available in Maldives
5) Thalassaemia screening is mandatory for marriage registration in Capital, Male'

All services provided at National Thalassaemia Centre are free of charge. Budget is provided by the Ministry of health.

Thalassaemia Statistics:
- No of registered patients at NTC till April 2011: 726
- Total no. of patients receiving treatment: 537
- Total no. of deaths till April 2011: 157
- After registration did not report to NTC: 32

National Thalassaemia Centre Blood Bank

It provides blood to all thalassaemic patients after screening blood for TTI Markers and checking for compatibility.

Source of blood at National Thalassaemia Centre Blood Bank is
- Collection of blood at National Thalassaemia Centre Blood Bank by Voluntary and few directed donors.
- Donation of blood from National Blood Transfusion Services (NBTS)
- Donation of blood from Indra Gandhi Memorial Hospital (IGMH)

CURRENT SITUATION IN CONTROL STRATEGIES AND HEALTH SYSTEMS IN ASIA, NEPAL

Ajit Rayamajhi

Associate Professor of Paediatrics, Department of Paediatrics of National Academy of Medical Sciences in Kanti Children's Hospital, Kathmandu

Abstract. Nepal lies between India and China and has a population of 30 million. The National Health Policy was adopted in 1991 with the objective of extending primary health care system to the rural population so that they benefit from modern medical facilities and trained health care providers. At present, Second Long Term Health Plan (1997- 2017) developed by Ministry of Health provides guiding frame work for the development of annual health plans, appropriate strategies, programmes and action plans that reflect national health priorities. A *National Policy on Non Communicable Diseases (NCD) Control and Prevention* is being formalized soon with a goal to reduce morbidity and mortality related to NCD including thalassaemia. NCD contributes to 42% of total deaths. The strategies adopted are to develop and endorse legislation for patient right, promote advocacy and community mobilization, establish effective disease surveillance including risk factors through Health Management Information System reporting, establish network and develop mechanism to monitor activities of different government and non government organizations working in NCD, prioritize cost effective socio-culturally acceptable measures in planning and implementation, allocate regular budget and local revenue for preventive activities, develop appropriate human resources, produce effective tool for monitoring and evaluation and conduct periodic research activities based on it. The support of blood and blood products for patients of thalassaemia has been since 1966 with the establishment of a *Blood Bank* by Nepal Red Cross Society. It now operates based on *The National Guidelines on Management of Blood Transfusion Service* developed in 2008. The welfare of patients of thalassaemia is looked after by a charity organization the Nepal Thalassaemia Society which was established in 2003. It maintains records of patients of thalassaemia, provides blood for transfusions free of cost, facilitates provision of chelating agents, conducts blood donation camps and manages a blood transfusion center.

THALASSAEMIA IN SRI LANKA

Rasnayaka M. Mudiyanse,[1] Ashok Perera,[2] Dayananda Bandara[3]

[1]Department of Pediatrics, Faculty of Medicine, University of Peradeniya, [2]Post Graduate Institute of Medicine, University of Colombo [3]National Thalassaemia Center, Kurunegala
Email: rasnayakamudiyanse@gamil.com

Abstract. Sri Lanka, a 65,610 km^2 size island located south of India has 20,653 million people, enjoying an average life expectancy of 75 years, literacy ratio of 92%, vaccination coverage 90%. Child Mortality Rate (CMR), Infant Mortality Rate (IMR), Neonatal Mortality Rate (NMR) were 10.4, 8.5 and 5.9 per thousand live births respectively in 2006. Maternal Mortality Rate(MMR) in 2007 was 14.2 per 100 000 births. Per capita income is 2399 USD per year.[1,2] We managed to trace1678 thalassaemia patients in the entire country. Estimated incidence based on age frequency data is 60-65 /year. Carrier rate vary from 0-5%. All the patients are provided unrestricted Leuco reduced blood free of charge. Blood filters are yet to be introduced. Poor transfusion compliance is caused by poverty. Quantity of chelators used is less than 50% of ideal corresponding with 50% of patients having serum ferritin above 2500 ng/l and life expectancy ranging from 10 -15 years. Thalassaemia prevention was initiated recently; 15 years starting palliative treatment. Voluntary screening by FBC is offered for 15 years and above. Subjects with MCV <80 fl and MCH <27 pg are given iron treatment for 3 months. Those with persistent low MCV and MCH are given a pink card after confirmation by HPLC. Non carriers are given a green card. Already National Thalassaemia Center has arranged to issue 5930 pink cards after screening nearly 75, 000 people. Marriage between two thalasaemia carriers is risky expressed as mismatched thalassaemia horoscope. Committed doctors educate public voluntarily, awaiting an organized campaign. Thalassaemia is yet to be introduced in to school curriculum. Monitoring prevalence, incidence, high risk marriages, high risk pregnancies and screening coverage are expected to be established soon. Developing consen-

sus, recognition of priorities and allocation of funds has become a challenge. Thalassaemia prevention should be integrated in to existing system.

Sri Lanka. Sri Lanka is 65 610 Km^2 size island south of India. Central hilly area is surrounded by flat lands. Total population is 20,653 million. The country belongs to low income category. However during last two years annual per capita income has improved up to 2399 USD. There are nine administrative provinces and each province is sub divided in to several administrate districts. There are 25 such districts in the country. Each district is divided in to Grama Seva (GS) divisions of public administration to implement national government policies. Each Grama Seva niladari looks after 500 – 1000 families. There are nine Provincial Directorates of health services(PDHS), with 291 Medical Officer of Health (MOH) divisions headed by graduated medical officer. Each MOH division has 15- 40 Public Health Midwife (PHM) who visits all the houses and promotes all health care activities. There are 43 PMH per 100 000 population. All the births and marriages in the country are registered under vital statistics by the department of registrar general. There were 364, 565 births and 200, 985 marriages registered in 2010. A registrar of marriage usually registers about 500 – 1000 marriages per year. The average life expectancy for male is 68.1 and female is 76.6 years (2000 -2002).[3] Literacy ratio of 92% is due to well established primary and secondary education in the country. Vaccination coverage is satisfactory; reaching 90%. Sri Lanka has already achieved most of its MGD goals; Child Mortality Rate (CMR), Infant Mortality Rate (IMR), Neonatal Mortality Rate (NMR) for the year 2007 was 10.4, 8.5 and 5.9 per thousand live births respectively. Maternal Mortality Rate (MMR) in 2007 was 14.2 per 100 000 births.[4] All the mortality indicators show a downward trend. Health care delivery is almost equally contributed by the government and private sector. Private sector contribution is mainly for therapeutic services rather than prevention and health promotion. However management of thalassaemia is entirely provided by the state and almost all the patients are treated in government hospitals. Thalassaemia preventive activities would be launched by its well established preventive sector.[1,2] Government of Sri Lanka has allocated 62, 910 million rupees (571.9 million USD); 3.2% of the total national budget for health in year 2011. Out of the total health budget 15, 000 million rupees would be allocated for drugs. Cost of chelating drugs for one year has been estimated to be around 150 million rupees.[2]

Thalassaemia prevalence in Sri Lanka. There are 83 hospitals with facilities for blood transfusion in the country. Out of them so far we have contacted 42 hospitals likely to cater for thalassaemic. Thirty hospitals had at least one patient. Distribution of these patients according hospitals, sex and diagnosis is given in the Table 1. Majority number of patients are treated at National Thalassaemia Center located at Kurunegala, where thalassaemia disease is highly prevalent in the country and large number of adult patients are looked after by Colombo North Teaching Hospital at Ragama. So far 1678 patinets has been traced to respective hospital where they are been treated. Some of the patients were attending two institutions for treatment. Duplication was avoided by checking the name and date of birth of patients. Age frequency distributions of patients from 9 hospitals are compared with National Thalassaemia Center patients in Figure 1 and 2. Average age among patients attending to hospital vary according to units. Overall average age is 13.4 years; average ages for beta thalassaemia patients are 13.3 and 12 for males and females respectively. However Hb E/ beta thalassaemia patients had a higher average age; 19.1 and 16.3 for males and females respectively. Life expectancy of thalassaemia patients seems lower than what was observed by a similar study in Italy.[5]

Table 1. Distribution of number of patients and average age according to diagnosis and institutions. (data from 9 hospitals available. Total number of patients from other hospitals is given below*).

Institute	Total	Avg age	Male						Female					
			Total	Beta Thal Major	Beta Thal Major Avg	E beta Thal	E Beta Thal Avg	Other	Total	Beta Thal Major	Beta Thal Major Avg	E beta Thal	E Beta Thal Avg	Other
NTC - Kurunegala	630	16	318	215	13.3	83	19.6	20	312	188	12.7	99	23.2	25
Anuradhapura	208	11	99	68	10	28	13.2	3	109	88	9.5	16	15.1	5
Badulla	89	11	44	33	11.3	7	12.1	3	45	41	10.2	3	9.7	1
Chilaw	55	16	35	25	14.4	7	19.3	3	20	12	16.9	5	16.8	3
Ragama	166	27.8	87	46	20.1	14	26.6	17	79	33	22.5	22	25.7	24
Kandy	71	13	28	17	13.4	6	14	3	43	33	11.4	2	17	8
Peradeniya	43	11	19	15	14.7	1	17	3	24	22	10.3	2	15	0
Monaragala	32	6.1	9	9	7.3	0	0	0	23	22	5.7	0	0	1
Hambanthota	12	6.9	6	3	7	1	0	2	6	5	6.6	1	8.5	0

*Institution and number of patients- Polonnaruwa 44, Lady Ridgeway Hospital for Children Colombo 80, Amparai 56, Jafna 2, Galle 35, Trincomale 20, Vavaniya 7, Mannar 1, Batticolre 38, Mahiyanganaya 5, Puttalm 6, Rathanapura 6, Kegale 6, Other hospitals and medical unites 65, Total number of patients 1678.

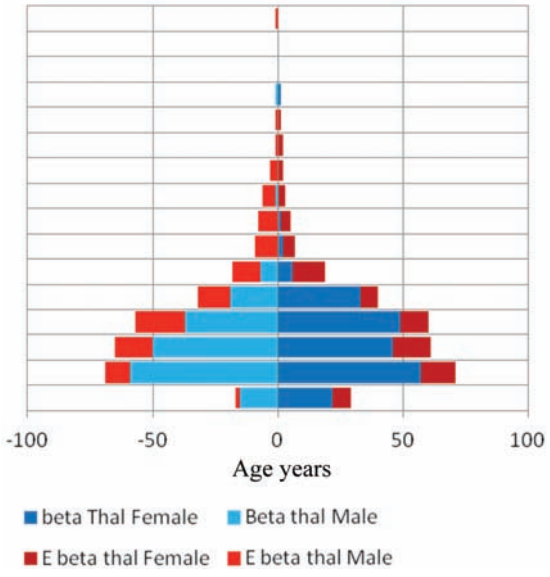

Figure 1. Age frequency distribution of beta thalassaemia and E beta thalassaemia patients in NTC, Kurunegala. Each bar represent 4 year interval starting from 0-4 yr, up to 48-52 yr.

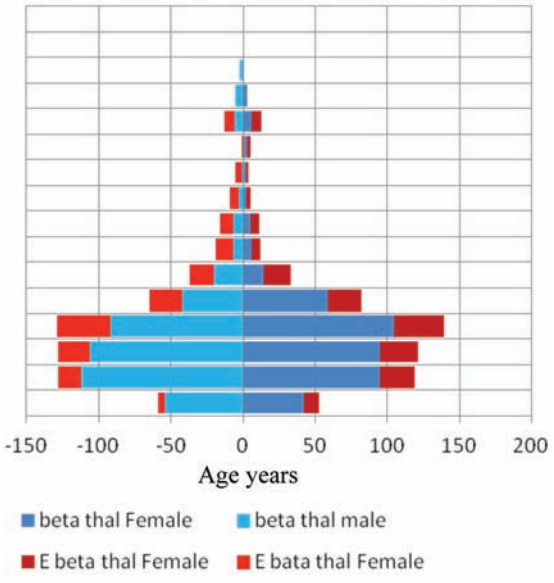

Figure 2. Age frequency distribution of thalassaemia patients in Sri Lanka

Average gene frequency of thalassaemia has been assessed according to districts. Gene frequency vary from 0-5% for beta thalassaemia and 0-3% for e beta thalassaemia. Wayamba (North Western) Province has a gene frequency of 0·0125 for beta thalassaemia and 0·0075 for HbE.[3] Calculations done using Hardy Weyn Burge formula based on the population of the province of 1·5 million, it has been estimated that there should be about 220 patients homozygous for beta thalassaemia and 260 patients with HbE/beta thalassaemia living in this region.[3] Estimated total number of beta thalassaemia patients for the entire country was 2350 and Hb E/Beta thalassaemia was 1060.[3] However the actual numbers of patients are much less than this estimate, suggesting the probability of avoiding at risk marriages by the public. Incidence of thalassaemia carriers among people coming for screening to the National Thalassaemia Center(NTC) at Kururnegala is as high as 7-8% indicating the selective nature of the sample. Age frequency distribution suggests the incidence of thalassaemia around 250 new cases for 4 year interval, supporting previously reported annual incidence of 60-70.

Management of thalassaemia patients. All the thalassaemia patients are treated free of charge by the government hospitals. However the cost of transport and other expenses are too much for majority of the patients, resulting in poor compliance. They are entitled for a financial support given by the social service department, but that will cover only small fraction of their financial burden. Some of the volunteer organizations and donor agencies provide support but there is no regular or consistent program.

There is no documented national guideline for management of thalassaemia. In 2005 Sri Lanka collage of Pediatrician s(SLCP) appointed a committee to establish a national Guideline for management of thalassaemia patients but was not successful in completion of its targets. Currently, National Thalassaemia Center has its guideline and others get advice from NTC or follow the guidelines published by Thalassaemia International Federation (TIF).

Patient management. BLOOD TRANSFUSION. National Transfusion service ensures the availability of blood for transfusion free of charge. Safety of blood transfusion is assured by screening for Hep B, Hep C and HIV. Patients are admitted to wards for blood transfusion in monthly basis. They have to stay in the ward for 1-2 days. However this may extend to 2-4 days depending on delays in cross matching and blood availability. At present patients are on leuco reduced blood (LRB) and only a limited number of patients are provided with filtered blood. Universal use of filtered blood is delaying due to its cost. In spite of providing a free transfusion service compliance for blood transfusion is not satisfactory. Economic constrains and difficulties of hospital stay may be the reasons. Pre transfusion hemoglobin was below 6 gr in majority of thalasaemia patients attending to Badulla in a study done in 2005. Other institutions are similar. However patients attending NTC has better compliance for blood transfusion.

SPLEENECTOMY. Many patients undergo spleenectomy. Among patients in NTC 13.4% above 10 years has had spleenectomy. They get lifelong antibiotic coverage and necessary vaccinations.

CHELATION. All three chelators; Desfrrioximine (DFO), Deferiprone (DFP) and Deferasirox (DFX) are available for use. Quantities of drugs imported by the Ministry of Health (MoH) are given in Table 2. Desferrioxamine and Deferasirox are available to purchase from pharmacies, however it is rare for patients to purchase chelation drugs from pharmacies. Deferiprone is not available in private pharmacies. Number of average size patients that can be looked after by the quantity of drugs used is much less than the actual number indicating inadequacy of chelation (Table 1 and 2) Poor compliance of patients, irregularity of drug supplies, lack of written guidelines and inadequate motivation of the health care providers contribute for poor performances. Iron over load is assessed by the serum ferritin level. This facility is available for government hospitals during last two years. Average serum ferritin levels and number of patients having serum ferrtin levels above 2500 ng/dl vary according to their age group and institutions (Table 4 and 5). Patients attending NTC has higher average serum ferritin levels, and higher percentage of patients having serum ferritin levels above 2500 ng/dl. Two hundred and ninety eight out of 382 beta thalassaemia patients in NTC have serum ferritin level above 2500ng/dl. Out of these patients 134 are currently on Deferasirox.

MONITORING FOR COMPLICATIONS. All the patients are monitored for complications. Their growth, development and maturation are routinely monitored. Annual or biannual evaluation for diabetes by PPBS, hypothyroidism by T4 /TSH, Hypoparathyroidism by serum Calcium is done in all patinets. Cardiac involvement is assessed by ECG, CXR and echocradiagram, however facilities for MRI T2* or other methods of cardiac iron overload evaluation are not available. DEXA scans are available in Colombo but it is not used to evaluate thalassaemia patients. Delayed puberty is evaluated by hormone assay and treated with hormone replacement. Growth hormone assay and growth hormone replacement is possible but currently thalassaemia patients are not subjected to growth hormone assay.

Cost of thalassaemia management. Estimated cost of care for a thalassaemia patients vary from SLRs 300 000 up 400 000 (USD 2750 to 3650) per annum depending on the age and state of the disease.[10] Life time cost of care for one patient would be 10 million rupees. This cost is far too much for a country with a per capita income of only USD 2300. Majority of the thalassaemia patients are coming from low socio economic background. Therefore they depend on government supply of drugs and facilities. According to the current practice even though patients are provided with drugs they have to look after the cost of infusion pumps, travelling, syringes and needles. This is impossible for some patients. Such patients can ask for further financial support from the social service department or volunteer organizations such as Rotary or Lions clubs. However poor compliance among those resulting from socio economic reasons is very common.

Thalassaemia prevention. Importance of thalassaemia

Table 2. Number of DFO vials, PFP capsule and DFX tablets issued from the Medical Supply Division of the ministry of health Sri Lanka from 2006 up to 2011.

	2006	2007	2008	2009	2010	2011**
DFO 500 mg	299 924	262 314	293 193	368 246	296 576	148 782
DFP 500 mg	47 900	65 000	87 650	80 700	61 450	60 000
DFP 250 mg	35 400	55 000	78 250	86 750	35 000	20 000
DFX 400 mg	-	-	-	-	103 030	126 690
DFX 100 mg	-	-	-	-	4400	22 259
*	*1 060*	*956*	*1 087*	*1328*	*1065*	*571*

*Estimated number of 25 kg size patients could be managed using minimal doses of 20mg/kg/day for DFO and DFX and 75mg/kg/day for DFP. **Quantities of drugs issued only up to August 2011 are given in this Table.

Table 3. Number of DFO vials, PFP capsule and DFX tablets issued from the Medical Supply Division of the ministry of health Sri Lanka from 2009 up to 2011.

	2009	2010	2011**
DFO 500 mg	130 464	70 460	56 016
DFP 500 mg	10 000	-	-
DFP 250 mg	10 000	-	-
DFX 400 mg		36 840	66 840
DFX 100 mg		30 000	148 530
*	*450*	*360*	*424*

*Estimated number of 25 kg size patients could be managed using minimal doses of 20mg/kg/day for DFO and DFX and 75mg/kg/day for DFP. **Drugs issued up to September 2011 is given.

prevention has been highlighted by many from the time of initiating organized thalassaemia treatment around 1996. A provincial program for thalassaemia and iron deficiency prevention based on population screening by FBC has been proposed for Uva province of Sri Lanka in 2005 but did not progress.[6] Community based carrier screening has been delayed because of the uncertainty of a suitable screening method for the country. During this period possibilities of introducing osmotic fragility test or dye tests have been evaluated. HPLC as a screening test was considered to be too costly for country like Sri Lanka. Therefore, serious effort on establishing thalassaemia prevention has been commenced only 3 years ago 15 years after initiating a well organized treatment regimen. National Thalassaemia Committee was established in year 2008 with the objective of giving necessary technical advice on management of thalassaemia and thalassaemia prevention. Over the last two years National Thalassaemia Committee has established a screening protocol based on preliminary screening by MCV and MCH and confirmation by HPLC.(Annex one) Thalassaemia screening is done entirely on voluntary basis while obtaining a written consent. According to the National Thalassaemia Prevention Programme of the Ministry of Health, Sri Lanka, the whole island was divided into 4 catchment areas. Under those catchment areas 4 screening units were established in 2010. The screening unit which was established in NTC at Teaching hospital, Kurunegala is the most successful among all of them and it mainly covers Wayamba (NWP), which thalassaemia is highly prevalent out of all the provinces in the country.

Protocol for thalassaemia screening in Sri Lanka. Those who are above 15 years are offered FBC which is the screening test. The age group above 15 is selected because younger aged can have physiological microcytosis. Those who have MCV value more than 80 fl or MCH values more than 27 are given a green card. However if they have a family history of thalassaemia (homozygous or a heterozygous) or if they intend to marry a thalassaemia carrier or a thalassaemia patients they are offered HPLC as confir-

Table 4. Average serum ferritin levels according to institutions and age group.

Age Group	1 to 5		6 to 10		11 to 15		16 to 20		21 to 25	
Institute	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
NTC Kurunegala	2630	2918	3805	3240	4180	4515	4870	4251	5990	2810
Anuradhapura	1301	1458	3416	3099	3427	3920	2488	4022	1148	1677
Badulla	2666	2555	3003	3627	4022	3855	3816	3895	870	na
Kandy	2200	2467	2595	4370	4776	7977	na	na	na	na
Monaragala	3019	2077	4594	8479	5256	3580	na	na	na	na
Hambanthota	817	936	20000	3414	na	na	na	na	na	na
Chilaw	1000	3630	3482	2988	787	2241	3309	1128	1650	1881
Vavniya	1541	na	5808	2447	na	na	8963	0	na	na
Peradeniya	2543	2038	3109	3127	3830	2653	6165	987	-	-
Ragama	-	-	3240	1150	2709	3023	2737	3166	2522	2201

Table 5. Number and percentage of patients having serum ferritin levels above 2500 ng/dl.

Institute	Total live patients	Beta thalassaemia Total ferritin reports available	Beta thalassaemia Serum ferritin >=2500 (%)	E /Beta thalassaemia Total ferritin reports	E /Beta thalassaemia Serum ferritin >=2500 (%)
NTC - Kurunegala	630	382	298 (78%)	174	41 (24%)
Anuradhapura	208	156	90 (58%)	43	5(12%)
Badulla	89	74	65 (88%)	9	6(67%)
Ragama	173	70	27(39%)	33	5(16%)
Chilaw	55	34	18 (52%)	11	3 (27%)
Monaragala	32	19	15 (78%)	0	0
Kandy	71	47	41(87%)	6	4(67%)
Peradeniya	43	32	16 (50%)	5	3(60%)
Hambanthota	12	5	3 (60%)	0	0
Total	1313	819	573(70%)	281	67 (24%)

matory test. Those who are having MCV value equal or less than 80 fl or MCH values equal or less than 27 are treated with iron 6mg/kg/day for 3 month period after examination by a doctor. At the end of three month second full blood count will be done. Those who have persistently low MCV and MCH values will be tested by HPLC to confirm the carrier state. HPLC confirmed thalassaemia carriers are given a pink card. Those who have persistently low MCV and MCH values with negative HPLC results are referred to hematologists for further detailed evaluation. According to this protocol people with microcytosis have to wait for three months to get their results confirmed. Therefore, when results are urgently required it was decided to offer HPLC at the first visit. However they will be invited for a repeated HPLC and FBC count after 3 months of iron therapy. Repeating HPLC and the FBC was introduced to the protocol as a research because of the concern that some of beta carriers may give false negative results when they are iron deficient. Possibility of missing some e thalassaemia carriers as they may have normal range MCV and MCH values was a concern from the beginning of the screening program. However it was agreed that missing Hb E thalassaemia carriers are unlikely to cause a significant problems in the prevention program because combination of two Hb E thalassaemia carriers are unlikely to give rise to clinically significant thalasaemia disease. However a marriage of Hb E thalassaemia carrier with a beta carrier can produce an Hb E/ beta thalassaemia patient. This possibility was further minimized by screening all the proposed partners of beta carriers to check their proposed partners by HPLC in addition to FBC. Counseling and education tool called thalassaemia porondama is used as guide (Annex 2). Basic principal of prevention is based on teenage counseling in order to promote safe marriage while avoiding high risk marriages. The entire program is based on motivating the public to make a voluntary commitment for screening and avoiding a high risk marriage.

NTC has screened 74,128 and 1945 HPLC tests have been to identify 5930 carriers and counseled after issuing a pink card.

Marriages in Sri Lankan culture and reading horoscopes. Majority in Sri Lankan culture consider marriage as a sacred event that bring two families together rather than a private event between two persons. Family wedding is considered a very important event in life and it is celebrated spending lots of money. Partners for marriage are selected by boys or girls independently or with the support from their parents, friends or professional match makers. Socio, economic and cultural compatibility of a couple is highly considered for a marriage. Majority read the horoscope before finalizing a marriage, even though everybody does not adhere to recommendation given by the horoscope readers. Similar practices are observed in many countries in the region. Horoscope reading is widely practice in India, China and many other countries all over the world. Horoscope is a document prepared by an expert on the subject of astrology depending on the time of birth. It is believed that many events in life are influenced by the time of birth due to the position of planets. Suitability of the partner with regards to their sexual behavior, liking and attitudes is predicted by reading the horoscope. Thalassaemia horoscope has been designed to simulate the traditional horoscope. Reading this has been made simple by red color indicates high risk marriages and green color indicate safe marriages. (Annex two- thalassaemia horoscope).

Public opinion. Proposed thalassaemia prevention in Sri Lanka entirely based on voluntary participation of people. Therefore education and motivation become essential. General public endorse the strategies proposed by the National Thalassaemia Committee. According opinion surveys done among university students, parents of thalassaemia patients and those who are coming for screening there is strong support for carrier screening, premarital counseling and monitoring marriages at the time of the marriage6. However significant number expresses concern towards antenatal diagnosis and abortions.

Future and monitoring thalassaemia prevention program. Thalassaemia management program in Sri Lanka needs monitoring and supervision. National thalassaemia register will be initiated soon. Registry for thalassaemic patients in a country is expected to serve as a tool for the development of cost-effective diagnostic and therapeutic approaches and for the definition of guidelines supporting the most appropriate management of the iron-chelating therapy and a correct use of the available iron-chelating agents.[5] Thalassaemia prevention program of Sri Lanka involve three grass root level officers; Public Health Midwife, Grama Seva niladari and Registrar of Marriages. Medical officer of health supervise 30-50 PHM divisions. Professionals involved in thalassaemia prevention need further education and training regarding thalassaemia prevention and genetic counselling.[7] Incidence of high risk pregnancies could be monitored at regional level as almost all the mother does register in an antenatal clinic. Similarly at risk marriages could be monitored by the registrar of marriage. Monitoring the screening coverage by the public health midwife would be a very valuable tool for monitoring at the beginning of the prevention program. Public health midwife visit all 500 – 1000 house hold at least once a year to promote health care policies of the ministry of health. Maintaining an eligibility register and regular update of screening coverage would be an essential policy to initiate a thalassaemia prevention program in the country. Introducing theses monitoring indicators are awaiting policy decisions by the ministry of health Sri Lanka. Thalassaemia prevention strategies need to operate forever in the country. Therefore, it is essential to select most cost effective and socially acceptable methods. Integrating all the functions of thalassaemia prevention in

Table 6. Thalassaemia screening which was done by thalassaemia screening unit of NTC till August 2011.

Details	Total	HPLC
NTC Daily Clinic Blood Test	32858	10611
Field Screening Blood Test	33155	2084
Hospital Ward Blood Test	344	47
PD office Blood Test - HM	–	1084
Blood Test before implementing protocol	7771	1945
Total Blood Test	*74,128*	*15,771*

the existing infrastructure and system would ensure sustainability of the program. High cost segregated program are likely to fail due financial constrains. Main emphasis of the thalassaemia prevention should be on education of the public enabling them to make an informed decision. Need to legalize abortions for the prevention of thalassaemia is been discussed, at times underestimating the value of current screening program underway. However resistance from religious groups and public against abortions is likely. Such process is likely to take several years. Introducing the screening and counseling teenagers is expected to bring down incidence of thalassaemia significantly and it would be the best strategic approach for the movement. Scientific observations can guide the public and politicians for future decisions regarding the need for introduction of antenatal diagnosis and abortions as another option available for public.

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THE THALASSAEMIA SITUATION IN AUSTRALIA

Sant-Rayn Pasricha, Don Bowden

Victorian Thalassaemia Services, Southern Health, Victoria, Australia

Abstract. Thalassaemia in Australia reflects the nation's history as a country of migrants. In 2010, 27% (6 million) of the Australian population (22.3 million) was born overseas. Persons born in China (1.7%), India (1.5%) and Italy (1.0%) comprise the largest non-UK/New Zealand born population, and over 2 million Australians claim Chinese, Indian, Greek, Italian or Vietnamese heritage. As a result, a broad spectrum of haemoglobinopathies is seen. Facilities for general haematology, haemoglobin electrophoresis and HPLC are routinely available in clinical laboratories, whereas only specialised laboratories perform genetic testing; Victoria has a single state service. Guidelines from learned societies recommend that obstetric units define a local policy for antenatal screening for haemoglobin disorders based on the local ethnic mix. Patients with thalassaemia are treated in every State; in Victoria there is a single specialist service, while elsewhere patients are cared for across multiple centres. Australia has the 10th highest GDP per capita worldwide; average earnings exceed $1000/week. Health expenditure comprises 9.0% of GDP; Australians have access to universal health coverage which provides access to free hospital, outpatient and primary care and investigations, subsidised medications and a national blood service which manufactures products exclusively collected from non-remunerated volunteer blood donors. Desferrioxamine, deferasirox and deferiprone are all available (with patient co-payment between AUD$5.60 and AUD$34.20/ month). MRI T2* cardiac and liver iron quantitation is becoming available to patients in some centres, and most patients have access to multidisciplinary care from haematologists, paediatricians, endocrinologists, gastroenterologists, social workers and other specialists. Future public health challenges will include adapting antenatal screening practices to suitably detect haemoglobinopathy carriers among descendants of an increasingly ethnically mixed population with high rates of intermarriage. Future clinical challenges will include the ongoing management of patients as they age beyond the seventh and eight decades.

Introduction. Australia is a migrant country with a diverse ethnic mix. As a result, a broad range of haemoglobin disorders is seen. In this paper, the Australian demographics and economy as they pertain to the epidemiology of thalassaemia will be presented. The Australian health system will be outlined, including provisions for access to medications (including iron chelation) and the national blood service. The best estimates of the prevalence of haemoglobinopathies in Australia will be presented. The services available for screening for and treatment of haemoglobinopathies will be indicated. Finally, challenges for the future will be postulated.

Methods. A review of information available from the Australian Bureau of Statistics (ABS), policies available from the Australian Department of Health and Ageing, and from the literature, together with consultation with health care providers treating thalassaemia around Australia, was undertaken.

Demographics of Australia. Modern Australia is a land of migrants, with the first settlers arriving from England just over 220 years ago. Of the over 22.7 million now living in Australia, about 27% (6 million) were born overseas; indeed, Australia has the third highest proportion of migrants anywhere in the world, after Singapore and Hong Kong. Australia's population growth has been achieved through migration; Australia's birth rate of 12.3 births per 1000 population (2010) is ranked 158th in the world, the current fertility rate is 1.9 per woman; indeed, births in Australia dropped below replacement levels as long ago as 1976. There were 297,100 births in Australia in 2008-09; in the same year, net immigration from over-

seas was 299,900 and comprised 66.2% of growth. Although the largest proportion of migrants arrived from the UK, of the 27% of Australians born overseas, 3.1% were born in Oceania, 3.7% in Southern and Eastern Europe, 1.5% in North Africa and the Middle East, 3.5% were born in South East Asia, 3% in North East Asia, 2.5% in Southern and Central Asia, and 1.3% in Sub-Saharan Africa. Persons born in China (1.7%), India (1.5%) and Italy (1.0%) comprise the largest non-UK/New Zealand born population; furthermore over 2 million Australians claim Chinese (670,000), Indian (234,700), Greek (365,100), Italian (852,000) or Vietnamese (174,000) heritage. Thus, a large proportion of the Australian population was born in or has ancestry from regions where thalassaemia and other haemoglobinopathies are prevalent. The Australian population chiefly resides in the major cities (about 15 million), including 4.6 million in Sydney, 4.1 million in Melbourne, 3 million in Brisbane. The concentrations of the overseas born population are highest in the capital cities, although communities are also seen in rural areas.[1] The Australian population continues to change: Australia has one of the highest rates of intermarriage between individuals of different ethnic backgrounds anywhere in the world. Of about 120,000 marriages recorded in Australia in 2009, about 42% involved at least one partner who was not Australian born. By the third generation, two-thirds of men and women of Chinese, Greek and Lebanese ancestries have partnered outside their ethnic group.[2]

The Australian health system. The average Australian income is approximately AUD$1020 per week. Australia has the 10th highest GDP per capita worldwide (approximately $1.23 trillion; $54,868 per capita).[3] Australians have access to universal health coverage (Medicare) funded through general taxation and a tax levy on higher income earners. This system provides access to free hospital, outpatient and primary health care. It also provides access to heavily subsidized pharmaceuticals (the *Pharmaceutical Benefits Scheme*), where patients pay a capped amount (currently up to $34.20, or $5.60 if the patient has a low income or has spent more than $1317 on medications in the current year) towards the total cost of the drug, with the Government paying for the remainder.[4] A parallel private health system, financed through private insurance, and which provides access to hospital and outpatient care, is available to the 51% of Australians who have purchased private health insurance.[5] Australia expends 9.0% of GDP on health (to both the public and private systems). This amounts to about AUD$113 billion annually, comprising $42 billion on hospitals (including $32 billion on public hospitals); $19.8 billion on medical services, and $15.2 billion on medications.[5] The Australian Department of Health and Ageing has a chronic disease policy that focuses on non-communicable diseases that are preventable and inflict the highest burden on the Australian population: these conditions include cancer, diabetes, asthma, cardiovascular disease, stroke, osteoporosis, osteoarthritis and rheumatoid arthritis. The principles of the policy include to adopt a population health approach and reduce health inequalities, to prioritise health promotion and disease prevention, to achieve person centred care and patient self management, to provide the most effective care, to facilitate coordinated and integrated multi-disciplinary care across services, settings and sectors, to achieve significant and sustainable change, and to monitor progress. The key action areas are mentioned as prevention, early detection and treatment, integration and continuation of prevention and care, and self-management.[6] This general approach could be applicable (but has not been formally applied) to patients with haemoglobinopathy/ thalassaemia.

Thalassaemia in Australia. The Australian population prevalence of various haemoglobinopathies has not been determined. It is likely that the carrier prevalence reflects the prevalence among the countries of origin of the Australians who have migrated to Australia from regions where disorders of haemoglobin are common. A systematic study of the prevalence of these conditions would be of value. There is no national policy mandating neonatal or antenatal screening for haemoglobinopathies. The Royal Australasian College of Obstetricians and Gynaecologists has recommended that each unit should have a defined policy for screening for haemoglobinopathies, taking into account the ethnic mix of patients screened. However, as a minimum, all women should be screened with MCV and MCH, with haemoglobin electrophoresis, iron studies and potentially, DNA analysis, performed in the event of thresholds not being reached. The College also suggests that testing of normal-MCV women for haemoglobinopathies should be considered if they are members of high-risk ethnic groups.[7] An approach to testing for thalassaemia has also been published in the Australian medical literature and is available from the Thalassaemia Australia Society.[8] Comprehensive diagnostic services for thalassaemia are available throughout Australia. Many routine public hospital and private clinical laboratories perform and report haemoglobin electrophoresis and high performance liquid chromatography. However, genetic testing for alpha thalassaemia and further confirmation of beta-chain mutations is only available in selected specialist laboratories. In Victoria, a single laboratory, funded as a State service, undertakes all referred genetic testing for genetic haemoglobin disorders. The laboratory has the capability to undertake a full range of genetic testing including multiplex PCR, restriction fragment length polymorphism, sequencing and multiplex ligation-dependent probe amplification. Prenatal testing of couples and antenatal testing of couples and the foetus is available in Australia. Foetal diagnosis through chorionic villus sampling is readily available. Abortion is legal if performed for severe foetal abnormality in all states of Australia other than Queensland, where it is legal if continuation of the pregnancy poses a threat to the health of the mother. In Victoria, any woman can access abortion until she is 24 weeks pregnant; abortion after 24 weeks is legal, but two doctors must agree the termination is appropriate, considering the woman's relevant medical circumstances and her current and future physical, psychological and social circumstances.[9] Couples at risk of thalassaemia may also opt for In-Vitro Fertilization with pre-implantation genetic diagnosis; this service is available but is associated with considerable out of pocket expenses for patients. There is currently no registry for patients with major haemoglobinopathy syndromes. Patients are treated in a single centre in Victoria, in two

centres in New South Wales, and in multiple centres in other capital cities. A survey of haematologists treating patients with thalassaemia indicates that there are about 326 patients with beta thalassaemia major in Australia and about 68 with sickle cell disease receiving transfusions. In Victoria, about 14% of patients with thalassaemia are children. Further work is needed to precisely quantify the burden of the thalassaemia and haemoglobinopathy (especially sickle cell) conditions in Australia and a national patient registry would be of value to this end. Patients with in Australia are able to join Thalassaemia Australia, the national consumer representative organization. National guidelines for iron chelation therapy have been recently published.[10]

Blood supply in Australia. Australia has a single national blood supplier, the Australian Red Cross Blood Service (ARCBS). The ARCBS collects, manufactures and distributes fresh blood products from voluntary donations. It also collects plasma for manufacture of specialized components and distributes these products to hospitals and other end users. Australia's blood sector is government funded, with cost-shared arrangements between the Federal (63%) and State (37%) governments, through the National Blood Authority which oversees and manages contracts with blood manufacturers.[11] All blood is collected from non-remunerated voluntary blood donors. Prevention of infection is achieved through stringent donor selection criteria and testing of all donations for HIV, hepatitis B and hepatitis C (serology and nucleic acid testing), syphilis (serology), HTLV (serology), malaria (in high risk donors), and CMV (at the time of first donation). Blood is now also screened for bacterial contamination, and transmission of variant CJD is minimized through the exclusion of former residents of the UK and individuals who have received transfusions in the UK from donating. In addition, red cells in Australia are now universally leucodepleted at manufacture. All patients receive blood products free of charge through the hospital system. Where required, washed and irradiated products are available.[12] Although the proportion of the total red cell supply used by patients with thalassaemia in Australia is unknown, a 2009 study found that 9.3% of red cells were transfused to patients with *non-malignant haematological conditions*.[13] The Victorian Thalassaemia Service is the single largest user of red cells in Australia. Transfusion therapy to patients with thalassaemia major is directed by local standard operating procedures; the treatment goal is to maintain a pre-transfusion haemoglobin between 90-110g/L, although this is individualised through consultation with patients based on their symptoms and the frequency to which they are able to attend for transfusions. Transfusion programmes are intensified when patients are pregnant or undergoing combination therapy for hepatitis C.

Iron chelation in Australia. Iron chelation therapy is available to patients in Australia through the Pharmaceutical Benefits Scheme. Desferrioxamine is restricted to patients with *disorders of erythropoiesis associated with treatment-related chronic iron overload*, deferiprone to patients with *iron overload in patients with thalassaemia major who are unable to take desferrioxamine therapy; or in whom desferrioxamine has been ineffective*, desferasirox to patients with *chronic iron overload in patients with disorders of erythropoiesis*. Each medication costs patients $34.20 (or $5.60 if concessional) per month.[4] In our centre, the majority of patients are now using desferasirox; a minority of patients remain on desferrioxamine due to intolerance or failure of desferasirox, or because of patient preference. Iron monitoring is available through ferritin testing and, over the last 12 months, MRI for myocardial and hepatic T2*. All patients in Victoria are currently being offered tissue iron measurement by MRI, and this technology is available in selected centres elsewhere in Australia. Iron chelation management in Australia is managed according to national consensus guidelines.[9]

Multidisciplinary care of patients in Australia. Patients with thalassaemia have access free of charge through the public system to comprehensive multi-disciplinary care, including access to medical specialties (endocrinology, bone and reproductive specialists; gastroenterology and hepatology; cardiology); medical investigations (laboratory testing, radiology including MRI in some centres, DEXA scans, echocardiography), and allied health including social work and audiology. In settings where there is a larger population of patients, this care is usually provided by selected specialists with experience in thalassaemia. Bone marrow transplantations services are accessible for younger patients although to date only a few procedures have been undertaken in Victoria.

Future challenges. There remain many future challenges facing the management of haemoglobinopathies in Australia. Pressing clinical challenges includes the ongoing management of patients as they age into their seventh and eight decades; in Victoria, the oldest patient recently turned 60. The interaction between ageing and thalassaemia (and iron overload) related cardiac and bone abnormalities (in particular) may present a new set of challenges for physicians treating this disorder. The most pressing public health challenge involves the heterogeneous but rapidly changing demographics of the Australian population. With ongoing migration from regions where haemoglobinopathies are prevalent (including an increase in migrants from Africa) and intermarriage between members of different ethnic groups, strategies to ensure prenatal or early antenatal identification of carriers may need to adapt. Finally, further work is needed to identify the epidemiology of thalassaemia/ haemoglobinopathy carriers and to determine the number of affected individuals in the Australia.

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CURRENT SITUATION IN CONTROL STRATEGIES AND HEALTH SYSTEMS IN ASIA, CAMBODIA

Prak Piseth Raingsey, Chean Sophal, Robyn Devenish

Director of the Department of Preventive Medicine, Ministry of Health, Cambodia

Though considerable efforts are currently being made to improve the situation, the state of Cambodian health is poor, child and maternal mortality is high and overall life span is reduced considerably compared with neighboring countries. The reasons for this are multi-factorial including poverty, food insecurity, limited health infrastructure and resources. It is a fact that anemia is a chronic problem in Cambodia, 60% of children are anemic with Hb < 105 g/L and 40% related to iron deficiency.

Method. The retrospective study used to gather all information on hemoglobinopathy among children under aged of 19 years. Nine provinces were randomized for performed studies to identify the Prevalence Rates of Hemoglobinopathies in Cambodia during the past 10 years (2000-2010).

Result. Several different methods were used by the various studies for Hb typing and Hb genotyping. Only two of the studies were specifically aimed at the prevalence of hemoglobinopathies and only four of the nine studies, tested for both α and β thalassaemia. Among 4,111 children only 53.34% have been tested on β-α thalassaemia and the other did not tested α thalassaemia. Whereas 41% of children have had hemoglobinopathie which were Hb H diseases 0.04%, Hb E homogeneous 17%, Hb E heterogeneous 29%, Hb E trait 20 %, Hb E/ β thalassaemia 13%, β thalassaemia 0.01%, α thalassaemia 20.95%.

Discussion. In Cambodia the national policy or program on the prevention and control of thalassaemia has not yet formulated. Moreover the limited laboratory capacity to deal with the diagnosis of Hemoglobinopathies has considered as a major challenges, only CBC result and morphology has been done. Therefore only few pediatric hospital were able to do for Brilliant cresyl blue stain for Hb H bodies, Hb electrophoresis and DCIP (Dicholophenolindol) screening test for Hb E. However many cases go undiagnosed and untreated or misdiagnosed due to lack of local knowledge about thalassemia. Almost thalassaemia cases have had Hb <7.5 g/dl , the red blood cell transfusion is the only treatment available in Cambodia, but low availability. Iron and ferritin serum can be checked on patients who have received multiple blood transfusions. The Iron Chelator is not available in this country, and the majority of patient families have to find the blood themselves, the government has no policy to provide free blood transfusion. The national blood bank are not able to provide Leukocyte-Poor Red Cells for the thalassaemia blood transfusion.

Conclusion. Furthermore the urgent need to reduce the overall number of affected births and to improve the survival and quality of life of the patients with Hb disorders in Cambodia..

PREVENTION AND CONTROL OF THALASSAEMIA IN SOUTHERN CHINA

Chen Ping

Thalassaemia Research Institute, Guangxi Medical University, Nanning, Guangxi, P. R. China

Thalassaemias are the most common inherited disorder of hemoglobin synthesis in Southern China which includes the provinces of Guangxi, Guangdong, Hainan, Sichuan and Hunan. Their prevalence is about 20% in Guangxi and 11% in Guangdong which are the areas with the highest prevalence in China. Guangxi has a population of 50 million and it is estimated that about 3000 babies are born with thalassemia major annually. In Guangdong province, the total population is 80 million and the thalassemia major birth rate is about 1‰. Treatment of β-thalassemia major costs about 50,000 Yuan/year including blood transfusions and iron chelation therapy. Stem cell transplantation has been available since 1998, but it is not an option for most patients. Thalassaemias have therefore become a serious health problem in Southern China. In China, thalassemia research began in the 1960s. α-thalassemia genotyping and prenatal diagnosis were established and have been performed since 1983. Prenatal diagnosis for β-thalassemia started in 1987. After 40 years' research, the majority of the molecular bases of thalassemia in Southern China have been identified. The Chinese government is fully committed to the prevention and control of thalassemia. Guidelines for prevention and control of thalassemia have been developed. As a result of health education, screening for carriers, physician and medical worker training, and genetic counseling and prenatal diagnosis, prevention and control programs for thalassemia have been in place in Guangxi, Guangdong and some cities in the provinces of Sichuan and Hainan since the 1990s. The Ministry of Health of China and the Guangxi provincial government signed an agreement to support prevention of thalassemia in Guangxi Province In March

2010. The Guangxi government is therefore comprehensively promoting prevention measures for *Prevention and control program for thalassemia*. This program includes: i. Enhancing health education for students, couples and general public; ii. Setting up more centers and networks within the province for screening and diagnosis of thalassemia; iii. Training for medical doctors, nurses, technicians and health workers about management of the disease; iv. Thalassemia screening: provision of free screening to premarital or pregnant couples; v. Genetic counseling and prenatal diagnosis: establishment of more centers for prenatal diagnosis and provision of free tests for high-risk couples. Guangdong government also has conducted the *Maternal and Children's Safety and Health Project* for prevention and control of thalassemia and other diseases. The aim of these projects is to reduce the birth rate of thalassemia major in southern China.

CURRENT CARE OF THALASSAEMIA MAJOR PATIENTS IN HONG KONG

Vincent Lee

Division of Haematology, Oncology and Stem Cell Transplant, Department of Paediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hksar, China

Introduction. Thalassaemia is common in Southern China. In Hong Kong, the carrier rates of α- and β- thalassaemia are 5% and 3.4%, respectively.[1] With improved public education, antenatal care and prenatal diagnosis,[2] the incidence of new cases of thalassaemia major in Hong Kong has dropped dramatically. There are currently 363 thalassaemia major patients receiving regular blood transfusion in Hong Kong. All of them are under the care of the paediatric haematologists or medical haematologists in hospitals run by the Hospital Authority, the statutory body that provides almost free-of-charge health care services and is accountable to the Hong Kong Government.[3] There are approximately another a hundred patients who have received allogeneic stem cell transplantation and are now transfusion independent.[4] This article is to provide a synopsis of current care of thalassaemia major patients in Hong Kong

Demographic data of Kong Kong. Hong Kong is a special administrative region of the People's Republic of China. It has a landmass of 1,100km^2 covering Hong Kong Island, Lantau Island, the Kowloon Peninsula and the New Territories that include 262 outlying islands. Hong Kong has a population of 7 million and population density of 6540 people per square kilometer.[5] People of Chinese descent comprise the vast majority (about 95%) of the population. Other significant national groups include Indonesia, Philippines and Thailand. The annual birth rate is 11.4 per 1000 population. In 2010, the gross domestic product (GDP) was US$225 billion with GDP per capita US$32,000. The annual health and medical budget in Year 2010 was US$4.8 billion, this accounted for 16.1% of the recurrent government expenditure.

Health care system in Hong Kong. There are 12 registered private hospitals and more than 40 public hospitals in Hong Kong. The Hospital Authority (HA) is a statutory body providing public hospitals and related services to the citizens of Hong Kong. It also offers medical treatment and rehabilitation services to patients through hospitals, day hospitals, specialist clinics, general outpatient clinics, Chinese Medicine service and community outreach services that serve the whole of Hong Kong. It is running 41 public hospitals with 27,000 beds and 1.4 million discharges, 48 specialty clinics with 8.7 million attendances, and 74 general outpatient clinics with 5.2 million attendances per year. Hong Kong citizens can enjoy subsidised medical services that are provided by HA. The fee for acute hospital admission is currently US$6.4 per admission, hospital stay covering essentially everything is US$12.8 per night, specialist clinic US$7.7 per visit and general clinic US$6.8 per visit. Medications are charged at US$1.3 per item per 3 months as administrative fee. Special medications may be charged separately. Patients having financial difficulties however may have the charges waived, even for special medications, after social security assessment. The medical expenses in private hospitals are on the other hand either self-paid or covered by non-mandatory medical insurances.

Epidemiology of thalassaemia in Hong Kong. The carrier rates of α- and β- thalassaemia are 5% and 3.4%, respectively.[1] The commonest genotypes are (--SEA) Deletion (4.5%), Deletion of CTTT at codon 41-42 (1.2%), C→T at position 654 in IVS2 (1.0%), and Glu→Lys at codon 26 in the β-globin chain, Haemoglobin E (0.3%). Estimated number of affected pregnancies for α-thalassaemia, HbH and Hb Barts, to be 145 per year and β-thalassaemia major to be 80 per year. With the availability of prenatal and antenatal diagnosis, molecular diagnostic facilities and genetic counselling services, the number of new cases has dropped from a median of 16 per year to around 3 per year over a period of two decades.[3] These new cases are the results of missing antenatal check-up, declined prenatal diagnosis, antenatal check-up done outside Hong Kong where screening of thalassaemia is not routinely included, and wrong prenatal diagnostic results. As in 2010, there are 363 thalassaemia major patients in Hong Kong. The patients have a mean age of 23 (range, 1-52) years, and 78% of them are adults, 18 of whom are above 40 years old.3 There are another more than a hundred patients who have received allogeneic stem cell transplant and are now transfusion independent.[4]

Diagnostic services available in Hong Kong. Thalassaemia carrier state is not routinely screened in Hong Kong. However, when a person who incidentally found to have microcytosis, haemoglobin analysis can optionally be offered. While this has always been promoted, the majority of Hong Kong citizens have no pre-marriage and/or pre-conception medical check-up. In Hong Kong, universal prenatal screening for thalassaemia was started in mid 1990s. Pregnant women are universally screened for haemoglobin levels and the mean corpuscular volume (MCV). If microcytosis is found, the paternal MCV will then be checked. If both of the couples have microcytosis, iron profile and Hb pattern will be done. If both of them have blood results indicating thalassaemia trait, counselling will be offered. Genetic studies on common mutations/deletions are available in a number of public and private hospitals and laboratories in Hong Kong. If the molecular defect can be identified on both couples, prenatal diagnosis can be

done on foetal cells obtained by chorionic villus sampling or amniocentesis.

Management of thalassaemia in Hong Kong. MANAGEMENT TEAM. The Hospital Authority has a standardized management protocol, based on the TIF guideline, on diagnosis, transfusion policy, monitoring of iron overload, iron chelation therapy, and monitoring and management of complications. Multidisciplinary team approach has, however, not been widely practiced. Thalassaemia major patients had also traditionally been under the care of the paediatric haematologists, even after they have reached their adulthood. In 2010, a multi-disciplinary group including the paediatric haematologists, medical haematologists, radiologists and patient-parent representatives was formed with the aim to integrate the management of adult thalassaemia patients as well as to assist transition of patients from paediatric to adult thalassaemia centres.

BLOOD TRANSFUSION. Blood transfusion service is free of charge. Blood and blood components are obtained through the voluntary, non-remunerated blood donation programme operated by the Hong Kong Red Cross Blood Transfusion Service which is the only blood providing institution in Hong Kong. All transfusion dependent thalassaemia patients receive regular 3 to 5 weekly blood transfusion in the public hospitals. Pre-storage leukocyte-filtered, phenotype-matched blood units are pre-arranged for all patients one to two days ahead of their scheduled transfusions. The targeted pre-transfusion haemoglobin level is 9-10g/dL. In 2009, a total of 18,782 units of blood consumed, which account for 9.5% of all red cells collected in Hong Kong during the period.3

IRON ASSESSMENT BY MRI T2*. The development of a standardized MRI T2* assessment of body iron was a hallmark of thalassaemia management. As part of an international effort, the MRI scanner in Prince of Wales Hospital, the Chinese University of Hong Kong was calibrated against international standard in 2006.[8] Later, all patients with thalassaemia major in Hong Kong have been offered scanning with the support from the Hong Kong Children's Thalassaemia Foundation.

IRON CHELATION. All regularly transfused thalassaemia major patients receive iron chelation therapy. Desferrioxamine was made available in Hong Kong in 1970s and has markedly reduced the morality of thalassaemia major patients in Hong Kong, mainly due to the significant reduction in cardiac deaths.[6] Deferiprone was licensed in Hong Kong in 2005.[7] Deferiprone, either as monotherapy or in combination with desferrioxamine, has resulted in dramatic reduction in cardiac haemosiderosis and improvement of cardiac function, especially for those whose hearts have been heavily iron overloaded. Both desferrioxamine and deferiprone are now available in public hospitals free of charge. Deferasirox was licensed in Hong Kong in 2006. In mid 2011, deferasirox was made available free of charge in public hospitals to children younger than 6 years old and to patients who cannot tolerate the toxicities of desferrioxamine and deferiprone or who fail to achieve adequate chelation despite good compliance. Deferasirox is also available as a self-financed item to the other patients who wish not to receive the other two iron chelators. In 2010, the proportion of thalassaemia major patients receiving desferrioxamine alone, deferiprone alone, combination desferrioxamine and deferiprone, and deferasirox was 30%, 17%, 48%, 5%, respectively.

Complication and mortality. Cardiac failure is the most important cause of death in thalassaemia major patients. Among 180 patients surveyed in 2006, the prevalence of low ejection fraction (EF, <55%) was 19% with 34% of cases with history of heart failure.[9] The prevalence of endocrine dysfunction is also high.[3] Diabetes mellitus occurs in up to 25% of adults with thalassaemia and appears increasing. This may reflect inadequate chelation in early childhood. Hypogonadism is prevalent and half of all adult patients are on hormonal replacement therapy. A survey in 1999 showed that heart failure (65%), complications from haematopoietic stem cell transplantation (25%) and infections were the three leading cause of death of thalassaemia major patients.[6] An updated survey revealed that heart failure (61%), complications from stem cell transplantation (15%) and infections (10%) remained the main causes of death.[3] Encouragingly, the number of deaths has declined steadily over the past two decades (1996-2000, 33 deaths; 2001-2005, 16 deaths; 2006-2010, 10 deaths).

Our future. In Hong Kong, with the universal screen policy and the availability of prenatal diagnostic techniques, the number of new cases of thalassaemia major has dropped to a very low level. With better iron assessment and chelation therapy, life expectancy of thalassaemia major patients is expected to approach that of normal population. With the establishment of the joint paediatric-adult multi-disciplinary group, thalassaemia care is expected to be gradually shifted from paediatric to adult medical teams that not only involve the haematologists but also the radiologists, endocrinologists, cardiologists and social workers. However, the establishment of reference centres need to be explored. Only with close collaboration between the medical professionals as well as with the patient-parent groups, our thalassaemia patients will be able to enjoy a normal life with full education, successful career, marriage and family life.

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THALASSEMIA IN LAO PDR: DESK REVIEW AND KEY INFORMANTS INTERVIEW

Sourideth Sengchanh,[1] Alongkone Phengsavanh[2]

[1]Department of Pediatrics, Faculty of Medicine, University of Health Sciences, Lao PDR; [2]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Health Sciences, Lao PDR Assoc. Prof. Dr. Khampe Phongsavath, Sethathirath Hospital, Lao PDR

Aims and Objectives. The retrospective study aimed to describe the magnitude of thalassaemia by reviewing existing hospital based data and interviewing the policy makers and care providers about thalassemia treatment and prevention. The hospital data from central hospital in Vientiane were obtained from 2005 -2011.
Results. The number of Beta-thalassemia cases in Pediatrics wards of 3 central hospitals in Vientiane capital, were reported as 51 cases of beta-thalassemia major, 224 cases of Beta-thal/Bb E disease, 78 cases of Hb E trait, 29 cases of Alpha thalassemia. 37 cases of splenectomy, 177 cases of iron overload, 82 cases of iron chelation. Alpha thalassemia can be detected by sending blood specimen to Thailand, due to unavailability of detecting machine. In Vientiane capital, the prevalence of hydrops fetalis is 0.3%. The antenatal and pre-conceptional screening is not available including strategy and system. Some study about the thalassemia screening showed that the screening by using Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Osmotic Fragility test (OFT) and Dichlorophenol indophenol (DCIP) precipitating test are also feasible in hospital in Vientiane capital. After interviewing policy makers and care providers, some main outcomes are reported such lacking of human resources and medical equipment in the field of hematology, treatment guidelines are not unified, national strategy and screening are not available. Iron chelators are registered, but not register in the essential drug list.
Discussion and Recommendation. Development of national strategic planning for thalassemia treatment and prevention. Development of update national treatment guidelines for thalassemia. Initiation of national thalassemia screening program. Development of community and hospital based registration system for thalassemia. Inclusion of iron chelators into national essential drug list. Increasing the public awareness and community motivation regarding the cost-effectiveness for thalassemia prevention and treatment.

THE MALAYSIAN NATIONAL THALASSAEMIA PREVENTION AND CONTROL PROGRAMME

Hishamshah Mohamed Ibrahim

Senior Consultant Paediatrician and Senior Consultant Paediatric Haematologist & Oncologist, to the Ministry of health of Malaysia

Malaysia's country size is 329,959 sq. km with a multi-ethnic population of 28,334,135 (2010) and a population density of 86 per km^2. The Malays constitute 50.4%, Chinese 23.7%, Indians 7.1%, Indigenous people 11% and other ethnic groups 7.8 %. The majority of Malaysians (71%) live in an urban setting with a sizeable young population. An estimated 27.6 % are below 15 years old, 67.3% between 15 - 65 years old and 5.1% are above 65 years of age. The annual population growth rate is estimated at 2%, Crude Birth Rate was 17.9 per 1000, Crude Death Rate 4.8 per 1000, Infant Mortality Rate 6 per 1000 live births and Male, Female life expectancy of 71.7 and 76.5 years respectively. The average Income: GDP per capita (PPP) was US$414.400. The total expenditure on health as a percentage of GDP was 4.75% in 2010 and the total Ministry of Health allocation to the national budget is 8.02%. The health system is divided between the Public/Government and private sectors. The Government provides comprehensive healthcare from the primary to the tertiary care level while the private healthcare sector concentrates on urban-based tertiary care centres and independent solo-practice general practitioners. The doctor to population ratio is 1:859. The defining moment for Thalassaemia in Malaysia was on 25th August 2004, when the then Minister of Health who is a Medical doctor succeeded in getting a cabinet memorandum approved to initiate a national programme named The Thalassaemia prevention and control programme (Program Kawalan dan Pencegahan Talasemia). Prior to this, an estimated number of 2,400 transfusion dependent Thalassaemics (1995 hospital-based survey) received sub-optimal care without universal iron chelation. Although in general the quality of blood transfusion services is satisfactory, the majority of patients as expected succumb to complications of iron overload in the absence of adequate iron chelation in their late teens. No preventive component was in place and public awareness on thalassaemia was very low. The new national strategy includes a prevention component consisting of awareness campaigns and population screening, lab diagnostics of thalassaemia, provision of best-practice in clinical patient management and a patient registry. After 5 years, the scorecard from the national initiative was informative and showed improvement in many areas. In 2008, the Malaysian Thalassaemia Registry registered 3,588 patients but by 2010, the number has increased to 4,990 patients; beta-Thalassaemia major (44.7%), E-Beta Thalassaemia (31.6%), thalassaemia intermedia (9.8%), Hb H disease (10%) and other diagnoses (3.7%). The estimated carrier rate for alpha-thalassaemia was 1.8 – 7.5%, beta-thalassaemia was 3 – 5 % and Hb E was at 5 – 46%. Leucodepleted packed red cells was introduced for regularly transfused thalassaemia patients and iron chelation therapy was provided universally (Desferrioxamine 65.7%, Deferiprone 9.9%, Deferasirox 9.6 % and combination DFO+DFP 14.8%). Serum ferritin is still widely used as a surrogate marker for body iron stores and several local facilities also provide cardiac and liver T2*MRI assessment. The number of new thalassaemia births appears to be on a declining trend with antenatal diagnostic facilities being currently developed in selected centres. However selective abortions of affected fetuses are still not widely acceptable. There are still more to be done, but as demonstrated by our Malaysian model, a strong political will together with commitment from all stakeholders could dramatically transform and improve the care of our thalassaemia patients.

CURRENT SITUATION IN CONTROL STRATEGIES AND HEALTH SYSTEMS IN ASIA, MYANMAR

Ne Win

Myanmar

Abstract. Union of Myanmar has more than 57 million population and seven major indigenous races and 135 minor ethnic groups. The annual birth rate is 2.3% and there are estimated newborns who will be transfusion dependent thalassemia major ranges from 1300 to 6500 every year. Hence carrier rate of alpha thalassemia is 10-14%, beta thalassemia is 0.8-1.7% and hemoglobin E is 10-30% varying with ethnic groups. Health care system of Myanmar includes community cost sharing and free of charge system as of individual affordability. Both private and public sector of health are under Ministry of Health. Diagnostic service facilities for haemoglobinopathies are available only at the central and national level hospitals. Molecular diagnostic facilities are available only at the National Health Laboratory (NHL). Patient registries are completely placed in tertiary care hospitals and day care centers. Iron chelators are used only at the tertiary care hospitals and some central hospitals where specialists and diagnostic facilities are there. There is no prevention and control programme in Myanmar at all levels and sectors at the moment.

Background. According to annual hospital statistics report (2008), there are 4079 thalassaemic patients (unspecified) are admitted to hospitals in Myanmar, 1969 males and 2110 females; more than 2000 were children (<12 year of age). A minimum of 2-3 times of blood transfusion are needed annually individually. Myanmar government spent 65 billion Kyats for health in 2009-2010, one third of which is for curative and rehabilitative services. Estimated population of Myanmar is 59.13 million in 2009-2010 and 800,000 live births, 800 – 4000 newborns / infants will be born with lifelong transfusion dependent thalassemia.

Recent findings. During 2010, 1750 children attended Day Care Room of Yangon Children Hospital, 110-150 cases every month, to undergo packed cell transfusion. One of the transfusion requirement is PCV <18%. One third of the patients were less than one year of age at the time of diagnosis, another one third between 1 – 3 year of age and the last one third are more than three year of age. About 80% of the total blood transfusion is supplied directly from the hospital blood bank and the remaining from own donors. One unit of blood costs 9500 Kyats to the government for blood grouping, collection, storage and TTI screening tests (HIV, HBV, HCV, and Syphilis) and provided the patient free of charge. Thalassemia is more common in middle income and low income families. One visit to the DCR to undergo transfusion costs the family 17500 Kyats about two-third of which were non-medical costs like traveling and meals; a regular daily income may be lost in some cases; A financial burden to the family and also to the government. Importantly, there is a need a nationwide prevention program for thalassemia / haemoglobinopathy.

Suggestion for future work. No law enforcement like legal abortion for thalassemia major deters the development of National Prevention Program. However awareness raising to the community through health education programs, premarital counseling carrier screening, risk couple screening must be encouraged. Psychosocial problems of the patients need to be explored and must be treated and prevented. Ways and means of reduction of financial burden to the family and to the government should be explored through health system research and health economy research.

ABSTRACT ON COUNTRY REPORT, PHILIPPINES

Ernesto d'J. Yuson

Thalassaemia Centre of the Philippines, Haematology - Oncology Unit, Dr. Fe del Mundo Medical Centre Foundation Philippines, Quezon, Philippines

The Philippines is situated at the upper part of Southeast Asia rooted on a 7,100 islands separated by body of water, mainly rivers, lakes, seas and bounded by the Pacific Ocean on the eastern side and the Philippine sea on the west. At present, there are about 92 to 95 million Filipinos (extrapolated from the 2007 data of 88.5M), although about 8 – 10 million are living abroad either for overseas work or immigration to different parts of the world. Awareness and knowledge on the Thalassemias and hemoglobinopathies are just in its infancy in the overall local medical parlance, since the technology on determination and diagnosis came very late in the country (electrophoresis in 1985 and HPLC in 2008). The technology on red cell indices determination are likewise not standard or uniform in all institutions. Much the same way, the medical professionals earlier believe that thalassemia was not present in the Philippines, until awareness increased and cases of transfusion dependency becomes evident. The government has no national program on heritable diseases like thalassemia, because their focus is still on prevention, control and treatment of infectious diseases and cancers. More so, there seemed to have no allotted budget for thalassemia per se. At present, both alpha and beta thalassemias are seen and reported among Filipinos and the major impact are seen among quite a few hundreds of beta thalassemia if at all. To date, there is no national health program to cover the overall expenses of a thalassemic, except for the Philippine Health Insurance System which can only provide a percentage of the therapeutic expenses but not the diagnostics; and support from their own families. Personal and private health insurance usually deny a patient's claim once they come to know that the disease is hereditary. Likewise, there are no blood allotment for thalassemics, despite there is an existing national blood program. Blood products are mainly derived from voluntary donotion system initiated by the government through a law passed in 1997 known as the National Blood Services Act. It was extrapolated that only about 3,000 to 5,000 packed red blood cell units (200ml/unit) are utilized for thalassemics per year at the moment. Thalassemia characterization is still underway through very limited research-oriented laboratories, yet fewer offer genetic analysis and molecular characterization. Treatment of disease consequence or transfusion-related complications are available (anti-virals and iron chelators), except not covered by government or private insurance system. Lastly, it is ideal and recommended that the

government should be able to come up with a national program on thalassemia determination, prevention, control and treatment.

CURRENT SITUATION IN CONTROL STRATEGIES AND HEALTH SYSTEMS IN ASIA, SINGAPORE

Law Hai Yang

Deputy Director, National Thalassaemia Registry, KK Women's and Children's Hospital, Singapore

Singapore is an island country with a population of 5 million with 74.1% Chinese, 13.4% Malay, 9.2% Indian and 3.3% of other ethnicities. The National Thalassaemia Registry (NTR) was set up in May 1992 with the support of Ministry of Health to register all individuals with thalassaemia and to offer free counselling and subsidized screening to their first degree relatives. It is currently housed in KK Women's and Children's Hospital (KKH), a government restructured hospital and a referral centre to provide healthcare to women and children. A standard notification form is used by doctors to refer index cases upon obtaining patient's or parent's consent. Majority of index cases are referred from KKH. The rest are referred from 3 other major restructured hospitals, private hospitals and clinics, Singapore Arm Forces and patients themselves. The incidence of combined HbE and thalassaemia carriers is estimated to be 4.49%, with α-thalassaemia accounting for 2.91%, β-thalassaemia 0.93% and HbE 0.64%. Over the years, NTR saw an increase in number of patients screened. By end of 2010, NTR has collected information from 43471 index patients and their relatives. This is made up of 72.6% Chinese, 17.6% Malay, 4.8% Indian and 5.1% of other ethnicities, similar to the ethnic composition of Singapore population. Screening found 12595 of index's relatives to have normal status. A total of 30679 individuals were found to be thalassaemia carriers or having other haemoglobinopathies. Majority of them were α-thalassaemia minor (45.97%), followed by β-thalassaemia minor (37.58%), HbE trait (11.88%), concurrent α and β-thalassaemia carriers (1.15%). The number of β-thalassaemia major registered is 89 (0.29%); HbE with β-thalassaemia 101 (0.33%) and HbH disease 521 (1.70%). Only 3 families are carriers of HbS. Most, if not all thalassaemia major patients are followed up in 4 major restructured hospitals where subsidised treatment is available. We target to achieve a Hb level of 8 g/dL and above for all patients. Although all 3 iron chelators are available, most patients (≈90%) are on Desferrioxamine. The rest are on Deferiprone, Exjade or a combination of one of these with Desferrioxamine. Two laboratories in Singapore carry out genotyping and prenatal diagnosis for thalassaemia. Total number of prenatal diagnoses carried out in KKH in 2010 was 38: 15 for pregnancies at risk for β-thalassaemia major or HbE with β-thalassaemia, and 23 for Bart's hydrops fetalis. As a result of active screening and prenatal diagnosis, the number of new β-thalassaemia major children born was kept at minimal. With one birth each in years 2005, 2007 and 2009, and none in 2006 and 2008, a total of 3 new β-thalassaemia majors were registered in NTR since 2005.

CURRENT CARE OF THALASSAEMIA MAJOR PATIENTS IN HONG KONG

Vincent Lee

Division of Haematology, Oncology and Stem Cell Transplant, Department of Paediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hksar, China

Introduction. Thalassaemia is common in Southern China. In Hong Kong, the carrier rates of α- and β- thalassaemia are 5% and 3.4%, respectively.[1] With improved public education, antenatal care and prenatal diagnosis,[2] the incidence of new cases of thalassaemia major in Hong Kong has dropped dramatically. There are currently 363 thalassaemia major patients receiving regular blood transfusion in Hong Kong. All of them are under the care of the paediatric haematologists or medical haematologists in hospitals run by the Hospital Authority, the statutory body that provides almost free-of-charge health care services and is accountable to the Hong Kong Government.[3] There are approximately another a hundred patients who have received allogeneic stem cell transplantation and are now transfusion independent.[4] This article is to provide a synopsis of current care of thalassaemia major patients in Hong Kong

Demographic data of Kong Kong. Hong Kong is a special administrative region of the People's Republic of China. It has a landmass of 1,100km^2 covering Hong Kong Island, Lantau Island, the Kowloon Peninsula and the New Territories that include 262 outlying islands. Hong Kong has a population of 7 million and population density of 6540 people per square kilometer.[5] People of Chinese descent comprise the vast majority (about 95%) of the population. Other significant national groups include Indonesia, Philippines and Thailand. The annual birth rate is 11.4 per 1000 population. In 2010, the gross domestic product (GDP) was US$225 billion with GDP per capita US$32,000. The annual health and medical budget in Year 2010 was US$4.8 billion, this accounted for 16.1% of the recurrent government expenditure.

Health care system in Hong Kong. There are 12 registered private hospitals and more than 40 public hospitals in Hong Kong. The Hospital Authority (HA) is a statutory body providing public hospitals and related services to the citizens of Hong Kong. It also offers medical treatment and rehabilitation services to patients through hospitals, day hospitals, specialist clinics, general outpatient clinics, Chinese Medicine service and community outreach services that serve the whole of Hong Kong. It is running 41 public hospitals with 27,000 beds and 1.4 million discharges, 48 specialty clinics with 8.7 million attendances, and 74 general outpatient clinics with 5.2 million attendances per year. Hong Kong citizens can enjoy subsidised medical services that are provided by HA. The fee for acute hospital admission is currently US$6.4 per admission, hospital stay covering essentially everything is US$12.8 per night, specialist clinic US$7.7 per visit and general clinic US$6.8 per visit. Medications are charged at US$1.3 per item per 3 months as administrative fee. Special medications may be charged separately. Patients having financial difficulties however may have the charges waived, even for special medications,

after social security assessment. The medical expenses in private hospitals are on the other hand either self-paid or covered by non-mandatory medical insurances.

Epidemiology of thalassaemia in Hong Kong. The carrier rates of α- and β- thalassaemia are 5% and 3.4%, respectively.[1] The commonest genotypes are (--SEA) Deletion (4.5%), Deletion of CTTT at codon 41-42 (1.2%), C→T at position 654 in IVS2 (1.0%), and Glu→Lys at codon 26 in the β-globin chain, Haemoglobin E (0.3%). Estimated number of affected pregnancies for α-thalassaemia, HbH and Hb Barts, to be 145 per year and β-thalassaemia major to be 80 per year. With the availability of prenatal and antenatal diagnosis, molecular diagnostic facilities and genetic counselling services, the number of new cases has dropped from a median of 16 per year to around 3 per year over a period of two decades.3 These new cases are the results of missing antenatal check-up, declined prenatal diagnosis, antenatal check-up done outside Hong Kong where screening of thalassaemia is not routinely included, and wrong prenatal diagnostic results. As in 2010, there are 363 thalassaemia major patients in Hong Kong. The patients have a mean age of 23 (range, 1-52) years, and 78% of them are adults, 18 of whom are above 40 years old.3 There are another more than a hundred patients who have received allogeneic stem cell transplant and are now transfusion independent.[4]

Diagnostic services available in Hong Kong. Thalassaemia carrier state is not routinely screened in Hong Kong. However, when a person who incidentally found to have microcytosis, haemoglobin analysis can optionally be offered. While this has always been promoted, the majority of Hong Kong citizens have no pre-marriage and/or pre-conception medical check-up. In Hong Kong, universal prenatal screening for thalassaemia was started in mid 1990s. Pregnant women are universally screened for haemoglobin levels and the mean corpuscular volume (MCV). If microcytosis is found, the paternal MCV will then be checked. If both of the couples have microcytosis, iron profile and Hb pattern will be done. If both of them have blood results indicating thalassaemia trait, counselling will be offered. Genetic studies on common mutations/deletions are available in a number of public and private hospitals and laboratories in Hong Kong. If the molecular defect can be identified on both couples, prenatal diagnosis can be done on foetal cells obtained by chorionic villus sampling or amniocentesis.

Management of thalassaemia in Hong Kong. MANAGEMENT TEAM. The Hospital Authority has a standardized management protocol, based on the TIF guideline, on diagnosis, transfusion policy, monitoring of iron overload, iron chelation therapy, and monitoring and management of complications. Multidisciplinary team approach has, however, not been widely practiced. Thalassaemia major patients had also traditionally been under the care of the paediatric haematologists, even after they have reached their adulthood. In 2010, a multi-disciplinary group including the paediatric haematologists, medical haematologists, radiologists and patient-parent representatives was formed with the aim to integrate the management of adult thalassaemia patients as well as to assist transition of patients from paediatric to adult thalassaemia centres.

BLOOD TRANSFUSION. Blood transfusion service is free of charge. Blood and blood components are obtained through the voluntary, non-remunerated blood donation programme operated by the Hong Kong Red Cross Blood Transfusion Service which is the only blood providing institution in Hong Kong. All transfusion dependent thalassaemia patients receive regular 3 to 5 weekly blood transfusion in the public hospitals. Pre-storage leukocyte-filtered, phenotype-matched blood units are pre-arranged for all patients one to two days ahead of their scheduled transfusions. The targeted pre-transfusion haemoglobin level is 9-10g/dL. In 2009, a total of 18,782 units of blood consumed, which account for 9.5% of all red cells collected in Hong Kong during the period.[3] Iron Assessment by MRI T2* The development of a standardized MRI T2* assessment of body iron was a hallmark of thalassaemia management. As part of an international effort, the MRI scanner in Prince of Wales Hospital, the Chinese University of Hong Kong was calibrated against international standard in 2006.[8] Later, all patients with thalassaemia major in Hong Kong have been offered scanning with the support from the Hong Kong Children's Thalassaemia Foundation.

IRON CHELATION. All regularly transfused thalassaemia major patients receive iron chelation therapy. Desferrioxamine was made available in Hong Kong in 1970s and has markedly reduced the morality of thalassaemia major patients in Hong Kong, mainly due to the significant reduction in cardiac deaths.[6] Deferiprone was licensed in Hong Kong in 2005.[7] Deferiprone, either as monotherapy or in combination with desferrioxamine, has resulted in dramatic reduction in cardiac haemosiderosis and improvement of cardiac function, especially for those whose hearts have been heavily iron overloaded. Both desferrioxamine and deferiprone are now available in public hospitals free of charge. Deferasirox was licensed in Hong Kong in 2006. In mid 2011, deferasirox was made available free of charge in public hospitals to children younger than 6 years old and to patients who cannot tolerate the toxicities of desferrioxamine and deferiprone or who fail to achieve adequate chelation despite good compliance. Deferasirox is also available as a self-financed item to the other patients who wish not to receive the other two iron chelators. In 2010, the proportion of thalassaemia major patients receiving desferrioxamine alone, deferiprone alone, combination desferrioxamine and deferiprone, and deferasirox was 30%, 17%, 48%, 5%, respectively.

Complication and Mortality. Cardiac failure is the most important cause of death in thalassaemia major patients. Among 180 patients surveyed in 2006, the prevalence of low ejection fraction (EF, <55%) was 19% with 34% of cases with history of heart failure.[9] The prevalence of endocrine dysfunction is also high.[3] Diabetes mellitus occurs in up to 25% of adults with thalassaemia and appears increasing. This may reflect inadequate chelation in early childhood. Hypogonadism is prevalent and half of all adult patients are on hormonal replacement therapy. A survey in 1999 showed that heart failure (65%), complications from haematopoietic stem cell transplantation (25%) and infections were the three leading cause of death of thalassaemia major patients.[6] An updated survey revealed that heart failure (61%), complications from stem cell transplantation (15%) and infections (10%)

remained the main causes of death.[3] Encouragingly, the number of deaths has declined steadily over the past two decades (1996-2000, 33 deaths; 2001-2005, 16 deaths; 2006-2010, 10 deaths).

Our future. In Hong Kong, with the universal screen policy and the availability of prenatal diagnostic techniques, the number of new cases of thalassaemia major has dropped to a very low level. With better iron assessment and chelation therapy, life expectancy of thalassaemia major patients is expected to approach that of normal population. With the establishment of the joint paediatric-adult multi-disciplinary group, thalassaemia care is expected to be gradually shifted from paediatric to adult medical teams that not only involve the haematologists but also the radiologists, endocrinologists, cardiologists and social workers. However, the establishment of reference centres need to be explored. Only with close collaboration between the medical professionals as well as with the patient-parent groups, our thalassaemia patients will be able to enjoy a normal life with full education, successful career, marriage and family life.

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THALASSEMIA IN VIETNAM

Khanh Quoc Bach

Deputy Director of National Institute of Haematology and Blood Transfusion, Hanoi, Vietnam

Geography. Vietnam is located in the Southeast Asia, land area of 331,698 km2, bordered to the north with China, the western border with Laos and Cambodia, East and West-South Sea East. The population is about 87 millions, its growth rate is 1.2%. Life expectancy is 72.8 years. There are 54 ethnic groups, accounting for 85.7% Kinh. Buddhist is the most popular with 85%. Gross domestic product (GDP) reached about 104.6 billion USD, per capital income reached about $ 1,200 in 2010. The budget expenditures for the health sector is VND 30,055 billion in 2010 and VND 43,200 billion, accounting for 5.95% of total national budget, about 500,000 VND per person (approximately U.S. 25) in 2011. In Vietnam, there are both public and private including in Health system. Public system is divided into four main routes: (1) Central, (2) Provincial/city, (3) County/District and (4) Village (local level). There are three main fields: research, treatment and prevention that are performed at the institutes or leading hospitals. Private clinic concentrate mainly in large cities and on some of specialties.

Epidemiology. According to researches in some areas in Vietnam, the percentage of people carrying the alpha thalassemia gene from 1.7% to 25% , about 2.1% for average, the percentage of people carrying the beta thalassemia gene and HbE / β-Thal is 0.7% -20%, about 1.4% for average and the rate of HbE gene is 1.7% - 30%, 2.6% for average. Totally estimation of 5.3 million people carry thalassemia gene and more than 70,000 patients with thalassemia currently. Based on the gene frequencies presented here, approximately 1,050 babies will be born with Hb H disease and 450 with Hb Bart's Hydrops, about 430 with Beta thalassemia and 520 babies will be born with HbE/Beta Thalassemia.

Management. Thalassemia is a issue concerned by state and society that expressed by:

- The screening and counselling programme in six districts of Hoa Binh province
- Viet Nam Thalassaemia association was established in February 2011.
- Establishment of thalassemia treatment unit at the National Institute of Haematology and Blood Transfusion in August 2011.
- Many studies have been performed since 1960s.
- Thalassemia is in the list of diseases that are covered by Health Insurance for both in and out patients. .

Diagnosis and treatment service. Formula peripheral blood cells are applied in all the district, provincial and central hospitals. Hemoglobin electrophoresis can be done in some of provincial and all central hospitals. Define Thalassemia gene is performed in some specialized hospitals such as Children's Central Hospital, National Institute of Hematology and Blood Transfusion, Cho Ray hospitals. Prenatal diagnosis, fetal diagnosis just be done in Children's Central Hospital and Tu Du Hospital.

Treatment. Blood (red cell concentration) is transfused for thalassemia patients at district, provincial and central hospitals, but blood is not enough at some periods of year. Iron chelators (desferrioxamine and Deferiprone) are available at all Central Hospitals. Stem Cell Transplantation has been performed in Children's Central Hospital, Ho Chi Minh hospital of Blood Transfusion and Hematology. Blood and iron chelator are paid by Health insurance.

Action plan.

1. Building national Thalassemia programs (National Institute of Haematology and Blood Transfusion implement)
2. Establish Thalassemia centre in Hanoi, then deploy branches in the provinces which have high-frequency

gene carriers based on haematology and blood transfusion departments at the provincial hospitals (National Institute of Haematology and Blood Transfusion implement)

3. Screening and preventing programs in Hoa Binh province and the others (General Office for Population and Family Planning)
4. Provide training for health staffs at the provincial hospitals (National Institute of Haematology and Blood Transfusion and Vietnam Thalassemia Association)
5. Improving the quality of diagnosis and treatment
   5.1. Improving the quality of diagnosis
      - Develop diagnostic and treatment protocol uniformly across the country (Vietnam Thalassaemia Association and National Institute of Haematology and Blood
      - Deploying do haemoglobin electrophoresis in all provincial hospitals
      - Thalassemia genetic testing in the central hospitals
      - Doing prenatal diagnosis in more hospitals

   5.2. Improving the quality of treatment
      - Blood transfusion: trying to provide enough blood for Thalassaemia patients in all hospitals.
      - Perform phenotype blood transfusion for patients with red cell antibody
      - Iron chelating: put iron chelators into the list of essential medicines at the provincial hospitals.
      - Stem cell transplantation for patients with major beta thalassemia
6. Strengthening activities of Vietnam Thalassemia Association
   - Publication: Documents for patients/parents; for young; medical staffs
   - Training for active members
   - Education for patients/ family members
   - Set up operations to raise funds
   - Propagate to community
   - Marriage consulting is first priority
7. Desiring to be twinned with other Thalassemia centres and Thalassaemia association in the world.

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1st Pan-Asian Conference on Haemoglobinopathies

8-10 February 2012, Bangkok - Thailand

SCIENTIFIC PROGRAM

GLOBAL EPIDEMIOLOGY OF β-, HBE, α-THALASSAEMIA AND SCD - SPECIFIC REFERENCE TO ASIA

Vip Viprakasit

Department of Pediatrics and Thalassaemia Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Thalassemia is the most commonly inherited hemolytic anemia worldwide and due to the large population and high prevalence of thalassemia carriers, Asia populations account for the majority of overall thalassemia births. In Thailand alone, the frequency of α-thalassemia is 25%, and the World Health Organisation estimates that, without effective prevention and control programs, over 250,000 symptomatic patients will be diagnosed in this country over the next few decades. A similar scenario is predicted to occur in India, Sri Lanka, Indonesia, the Philippines and Malaysia. Hb E is the most common haemoglobin variant found in this region with a very high prevalence in the area adjointing Cambodia, Laos and Thailand known as Hb E Triangle with carrier prevalence up to 50%. Hb S causing Sickle cell anemia or β thalassemia/Sickel cell disease was commonly found in some region of Indian subcontinent, although only few indigenous individuals with Hb S trait has been identified in the main land of Southeast Asia.

The prevalence of various types of thalassemia and common hemoglobinopathies in Asia is summarised in Table 1*. In patients with severe thalassemia syndrome, although stem cell transplantation might be the treatment of choice in patients who have HLA-matched donors, however, such treatment has several drawbacks including limited availability of matched donors, cost of treatment, treatment related mortalities and morbidity and long-term consequences due to exposure of chemotherapy for patients' conditioning. Therefore, the mainstay of management for most patients consists of regular blood transfusions supplemented with iron chelation therapy to prevent the effects of iron accumulation. The aim of regular blood transfusion is to promote normal growth, allow normal physical activities, minimise transfusional iron accumulation, adequately suppress bone marrow activity and reduce cardiac overload due to chronic anemia. Hemoglobin levels maintained at 9 to 10 g/dl are thought of as optimal. Approximately more than one million thalassemia patients within this region will require such standard transfusion management, therefore this amount of disease magnitude will further become a major health resource burden and future health care cost for each Asian

Table 1*.

Country	Average carrier rate (%) for common thalassemia and Hb disorders				
	α^0-thal.	α^+-thal	β-thal.	Hb E	Hb S
India	<1	10-13	3-4	4-30	1-40
Sri Lanka	<1	13.6	1-5	0.5	<1
Bangladesh	NA	NA	2.4-8	2.3-18	1
Maldives	<1	28	18	0.8	<1
Myanmar	NA	10	1-5.3	4-48	NA
Thailand	2.2-9	8-30	1-3	10-50	<1[#]
Cambodia	1	15.5	2.8	10-54	NA
Laos	NA	11	5	> 30	NA
Vietnam	NA	3.5	4	10-20	NA
Southern China	15	1-5	1.7	NA	NA
Hong Kong	2.2	2.3	3.5	1-2	NA
Taiwan	5	2.3	1.1	<1	NA
Singapore	2-3	1-3	0.93	0.64	NA
Malaysia	4.5	16	4.5	1-3	<1
Indonesia	<1	3-20	3	1-33	NA
Philippines	5	2.2	1	NA	NA

*Modified from V. Viprakasit *et al.* Iron Chelation Therapy in the Management of Thalassemia: An Asian Perspectives *International Journal of Hematology* 2009; 90: 435-445.
N =not available, NR=no report, [#]; reported in a few index families.

country especially the cost of iron chelation therapy. Our recent study has shown that Asia-Pacific countries remain lack behind the rest of the world in term of chelation practice and majority of thalassemia patients in this region suffer from severe iron overload. With limited resource in most of Asian countries, it is important that a prevention and control program for severe thalassemia syndromes must be implemented to reduce the number of new cases and allocate limited health care budget to improve standard of care in existing patients. Understand the epidemiology of this common inherited globin gene disorder in a micro-mapping fashion from an appropriate population survey in all different regions will be important for any given Asian country to tailor their management strategy from appropriate treatment care service to a national prevention and control program for thalassemia. However, with the up-coming full ASEAN community establishment in 2015 with tentative increasing interaction and migration among people in this region, the whole region will become a global village. There is an urgent need to develop a global standard program for thalassemia screening, carrier detection and prevention and control strategy to provide a common platform to be widely applied.

OVERVIEW OF GENOTYPES/PHENOTYPES OF THALASSAEMIAS IN ASIA

Suthat Fucharoen,[1,2] Pranee Winichagoon,[1] Orapan Sripichai,[1] Thongperrn Munkongdee,[1] Saovaros Svasti,[1] Vip Viprakasit[3]

[1]Thalassemia Research Center, Institute of Molecular Biosciences; [2]Department of Biochemistry, [3]Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Thalassaemia diseases, both α-and β-thalassaemias, are clinically variable. Although the molecular defect of most thalassaemia is known, the severity of the patients who have seemingly identical genotypes can vary greatly. It is necessary to understand the "genotype-phenotype" interaction in order to get better understanding of disease severity to provide better care for the patients. Beta-thalassaemia/Hb E is one of the most common monogenic diseases in Southeast Asia. Clinically patients show remarkable disease heterogeneity, varying from nearly asymptomatic to severe transfusion-dependent disease in which the patients die within the first decade of life. Previous study showed that haemoglobin levels varied from 3-13 g/dl with mean hemoglobin 7.7+1.55 g/dl. Many genetic and non-genetic factors may contribute to this severity different in β-thalassaemic disorder. The primary modifiers include the broad spectrum of β-globin gene mutations. Those mild β^+-thalassaemia genes, with certain β-globin production, are less severe than those with β^0-thalassaemia. The secondary genetic factors include those involve in the imbalance globin chain synthesis in the red cells. The coinheritance of α-thalassaemia and genetic factors enhanced higher Hb F production is among the crucial genetic modifying factors. Recently we have conducted the GWAS in 235 mild and 383 severe β-thalassaemia/Hb E patients with the Illumina Human 610-Quad BeadChips array. Twenty-three SNPs in 3 independent genes were identified to be significantly associated with the disease severity. The highest association was observed with SNPs on the β-globin gene cluster (chr.11p15). The others were the intergenic region between the HBS1L and MYB genes (chr.6q23) and the BCL11A gene (chr.2p16.1). Association to the fetal haemoglobin level was also observed with SNPs on these three regions. Our data indicated that several genetic loci act in concert to influence Hb F levels of β^0-thalassaemia/Hb E patients. The heterogeneity in disease severity is also observed in haemoglobin H (Hb H) disease. The majority of Hb H disease results from double heterozygosity for α-thalassaemia1, due to deletions that remove both linked α-globin genes on chromosome 16, and deletional α-thalassaemia2 from single α-globin gene deletions (—/-α). However, Hb H disease may occur from interactions between α-thalassaemia1 with non-deletional mutations (αTα or αT) or with abnormal haemoglobins such as Hb Constant Spring, Hb Paksé, Hb Quong Sze, and Hb Pak Num Po. Patients with non-deletional Hb H disease are usually more anaemic with significant splenomegaly, and some may require regular blood transfusions and be even as severe as "Hb H hydrops fetalis". However, there is no clear genotype/phenotype correlation associated with this severe clinical syndrome since patients with identical genotypes do not necessary show the same severity. This suggests that other genetic and environmental factors play a role in modifying the degree of clinical severity in patients with non-deletional Hb H disease.

PREVENTION PROGRAMMES FOR HAEMOGLOBINOPATHIES – APPROPRIATE FOR ASIA – EXISTING AND NEEDED – POLICY AND LABORATORY PERSPECTIVE

Anuja Premawardhena

Sri Lanka

The world's most populous continent, Asia, contains within it countries with widely varying economic and social standards. Religions and social customs the populations practice within these countries too vary. These differences apply to the standards of living as well as the legal systems. The Asian region bears the brunt of the thalassaemic burden of the world. South and South East Asia alone has almost 50% of the world's carriers and almost half of all homozygote births occur within that region. Unfortunately the exact numbers of patients with the disease in most countries are not known. Though some countries have estimated numbers they too differ widely depending on the source. A better way to understand the burden of disease is by estimates of the number of births of thalassaemics, which are mostly derived from calculations. Yet again due to widely different prevalence of thalassaemia in different regions of the same country attempts to generalize from one study of a small population sample could give inaccurate results. This is especially so for countries with multiple ethnic groups living in different geographic locations. The lack of accurate data has a huge bearing on policy planning, as inaccurate data will not allow true esti-

mates of disease burden. Thus the first priority for any country embarking on a prevention programme is to have accurate data from micro mapping. The age old adage that "prevention is better than cure" couldn't be better suited in the case of thalassaemia. After all there is hardly a cure for thalassaemia and this is especially so from the point of cost to the nation. The two most widely used prevention strategies for thalassaemia are pre natal diagnosis (PND) with the option of termination of pregnancy (TOP) and dissuasion of marriages between two carriers of the disease. Whatever the final method used, an effective and sustainable programme of health education, carrier detection counseling and data management must be in place. The choice of the main strategy is decided by the stake holders based on the availability of technology, the legal system, and the perception of the public regarding PND and TOP. Though there are a few claims of success that programmes based on dissuasions of marriages are effective, their long term success is doubtful. Though every country may not achieve the success of the "Cyprus programme", it is vital when planning a national programme to know the limitations of the programme the country is embarking on. The planners should have a good understanding on the cost effectiveness of the technology used as well as the chances of success both in the short and long term. It is here that studying other programmes which have succeeded, as well as ones which have not, is of paramount importance. As thalassaemia prevention has to compete with hypertension, cancer, diabetes and cardio respiratory disease, the big four NCDs for health sector funding, it would be inadvisable for any health policy planners to be involved in non cost effective prevention programmes for thalassaemia. Thus there is a case for patience and deep study of ground realities before embarking on a large scale prevention programme.

GENETIC SCREENING AND PRENATAL DIAGNOSIS OF THALASSEMIAS AND HEMOGLOBINOPATHIES IN TAIWAN TODAY

Ching-Tien Peng, Su-Ching Liu, Chien-Yu Lin, Chang-Hai Tsai

Department of Pediatrics, Children's Hospital, China Medical University & Hospital, Taichung, Taiwan, Bureau of Health Promotion, Department of Health, Thalassemia Laboratory, China Medical University Hospital, Taichung, Taiwan, Department of Biotechnology, Asia University, Taichung, Taiwan

Taiwan is 100 miles off the southeast of China with a population of about 23 million people. The α-thalassemia carrier rate is about 4%, β-thalassemia around 2~3% and 15 to 20 hemoglobin variants have been found which are mostly stable with a few unstable types (hemoglobin C, E, & Tak). There are currently about 400 thalassemia major patients who receive regular therapy. Taiwan has had a national prenatal screening program for detecting thalassemias in pregnant women since 1993. There is an average of 350-400 fetuses screened in this way per year in 6 medical laboratories. The main diagnostic procedures are by DNA study of the samples obtained from chorionic villus or amniotic fluid cells. Between 1998 and 2011, prenatal diagnosis procedures for identifying thalassemias and hemoglobinopathies were performed on 1125 fetuses which were at-risk for α-hydrops and β-thalassemia major in one central Taiwan medical center. The data shows: 17% of the fetuses were at risk of α-hydrops, 4% for β-thalassemia major and 2 fetuses for β/E-thalassemia, resulting in early prenatal diagnosis and termination of pregnancies affected with homozygous α-hydrops and β-thalassemia major in this area. Ten percent of the 1125 fetuses were at risk of compound heterozygosity of β-thalassemia and an abnormal hemoglobin of the β chain, and 8 different β-thalassemia mutations have been found. Four mutations, IVS-II-654 (C>T), codons 41/42 frameshift (-TCTT), and nonsense codon 17 (A>T) and codon -28 (A>G), account for more than 95% of mutant alleles. Hb E [β26(B8)Glu→Lys, GAG>AAG] was found to be the most common Hb variant at about 0.5-1%. There are 3 genotypes of α(0)-thalassemia 1 and at least 6 of α(+)-thalassemia 2 in these specimens. The most common types of α(0)- and α(+)-thal were the SEA deletion and the -α(3.7) rightward deletion, with frequencies of 87.79 and 4.85%, respectively. The results of this study provide a reference for designing a locally relevant antenatal diagnostic test for controlling the spread of thalassemia. The program's success is indicated by the 70% reduction in the number of newborns affected with β-thalassemia major. Moreover, in order to reduce the choice of interrupting the pregnancy in case of affected fetuses, preimplantation or preconceptional genetic diagnosis has been set up for thalassemias in several centers of Taiwan.

TREATMENT OF PATIENTS WITH β-THALASSAEMIA

John Porter

University College London

The clinical management of thalassaemias can be considered under three concepts namely; prevention, treatment and cure. This lecture will focus mainly on clinical treatment aspects, while recognizing that prevention and cure are equally important goals. Thalassaemia syndromes vary in severity from mild to severe, and predicting the clinical course for intermediate cases is not always simple. Recent detailed descriptions of the clinical consequences in thalassaemia intermedia allow better definition of prognosis and intervention strategies in intermediate syndromes (Taher A, *et al*. Br J Haematol. 2011;152:512-23) (Taher AT, *et al*. Blood. 2010;115:1886-92). Management of thalassaemia major, with transfusion and chelation therapy has advanced substantially in recent years so that if the full range of chelating agents and tools for monitoring iron overload are available, and patients are treated at expert centres that the necessary infrastructure in terms of staff, then outstanding results can be achieved (Thomas AS, *et al*. Blood. 2010;116:[abstract 1011]). The risk of death from cardiomyopathy in TM has fallen considerably in recent years, so that other morbidities and causes of mortality are becoming increasingly important. Key question with modern chelation are to understand how low iron overload can be effectively reduced without the risks of chelator toxicities and whether endocrinopathies can be prevented with

the adoption of more ambitious chelation regimes. The use of MRI techniques (*e.g.* mT2*) has helped to identify those at the greatest risks of cardiomyopathy, so that intensive chelation treatment can be targeted for those most at risk: a recent audit of over 100 patients at UCLH and Whittington Hospitals, monitored with cardiac MRI for the last decade has shown that those with evidence of increased myocardial iron has fallen from 60% to 20% of patients. Furthermore, in this cohort, cardiac iron overload is no longer the leading cause of mortality (Thomas AS, *et al*. Blood. 2010; 116:[abstract 1011]). These patients received a range of chelation therapies including monotherapies of desferrioxamine, deferiprone or deferasirox or combinations of deferiprone and desferrioxamine, with about one third of patients switching therapies at least once. No clear difference in outcome was seen between different regimes, except that patients who had more than 2 switches of therapy were less likely to have achieved significant improvement in myocardial iron. The optimal treatment of milder forms of homozygote or compound heretozygote thalassaemias (thalassaemia intermedia) is less clear, due to the increased risk of red cell allo-imunisation in patients who begin transfusion relatively late in life. With the exception of small numbers of impressive cases, the response to HbF modulators in intermedia syndromes is unpredictable and thus far often disappointing. The risks and benefits of potentially curative therapy need to be balanced against the improving outcomes with non-curative therapy described above. Indeed while bone marrow transplantation from a healthy sibling donors has been the mainstay of curative treatment for over two decades, the extension of this approach to matched unrelated donors using high-resolution HLA typing has been associated with results in some centres comparable with those obtained employing an HLA-identical sibling, with estimated probabilities for obtaining thalassemia-free survival between 85 and 87%.(Gaziev, *et al*. *Bone Marrow Transplant*, 42 Suppl 1, S41, 2008). Furthermore, a recent successful report of an E/b 0-thalassaemia patient treated by gene therapy, shows that the applications and reach of curative therapy are likely to extend further. This latter approach (Cavazzana-Calvo, *et al*. *Nature*, 467, 318-322*l*) used heavy pre-treatment conditioning and a lentiviral vector that carried transcriptional control elements that were specific for red-blood-cell precursors. Two years after treatment, the patient is synthesizing 10-20% adult Hb. The long term safety needs to be established and the significance and safety implications development of a clonal expansion of haemopoietic cells bearing a vector insertion in the *HMGA2* gene, with significantly increased expression of HMGA2 need to be established.

TREATMENT OF BETA-THALASSEMIA INTERMEDIA

Elizabeth George

Department of Pathology-Unit Hematology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Serdang, Selangor Darul Ehsan, Malaysia

Introduction. Beta-thalassemia intermedia encompasses HbE beta-thalassemia the most common form of beta-thalassemia intermedia in the world. In Southeast Asia and in parts of Asia, HbE beta-thalassemia intermedia is more common than beta-thalassemia major. The term thalassemia intermedia (TI) refers to patients with clinical manifestations that are too severe to be minor and too mild to be major falling into an area "no man's land". Age of presentation in the absence of inter-current infection occurs after age of 2 years. Blood transfusion independency or infrequent are other key-points in the diagnosis. Thus, diagnosis by definition remains largely clinical. Knowledge of the molecular basis and pathophysiological mechanisms of the disease process of TI has increased in the last decade. Despite this, currently there are no clear guidelines for managing a patient with TI. The high variability of the clinical picture and lack of evidence based guidelines places the attending physician in a dilemma: *To treat or not to treat?*

Thalassemia intermedia: the disease process. The hallmark of the disease process is broadly classified into three: ineffective erythropoiesis, chronic haemolytic anemia and iron overload. Ineffective erythropoiesis from destruction of developing red cells in the bone marrow occurs as a consequence of imbalance in globin chain synthesis of hemoglobin (Hb). This together with peripheral hemolysis leads to chronic haemolytic anemia and hypoxia. The arbitrary mean Hb levels in normal children, adult females and males are 11, 12, 13 gm/dl respectively. In TI, the Hb level range is 5.5-11 gm/dl. *In the absence of blood transfusions, morbidity is high and complications due to the disease process are inevitable and increase with advancing-age*.

Clinical diverse phenotypes. The natural history has not been completely defined in many countries. Clinical variability of beta-thalassemia intermedia has been attributed to interplay of three mediating factors: gene modifiers, environment and access to health care facilities. Changing phenotypes are seen as a consequence of these three mediating factors. Most studies reported are on hospitalized patients and miss out on mild cases in the population. In addition, mild cases may be converted to transfusion dependency by regular blood transfusions in the absence of guidelines on treatment. In a number of countries in Asia and Southeast Asia, complete molecular studies on gene modifiers are not available.

Gene-modifiers. Identification of possible gene modifiers provides arbitrary guidelines of possible clinical phenotype and genotype phenotype correlation. Our understanding on these gene modifiers implicates two pathophysiological mechanisms. The first mechanism involves two modifiers (primary and secondary) that affect α and non-α globin chain imbalance resulting in ineffective erythropoiesis. The primary gene modifiers are specific alleles of thalassemia and the secondary modifiers are globin genes that affect output of specific globin chain synthesis. The second mechanism, the tertiary gene modifiers involve genes affecting the disease process but not globin chain production.

Table 1.1 Primary gene modifiers: Specific beta-thalassemia alleles

- β^0 / β^+
- β^+ / β^+ (homozygous, compound heterozygous
- β^0 / β^0 + alpha-thalassemia or HPFH
- Homozygous γδβ (homozygous)
- γδβ / β^+
- Dominant β-thalassemia
- Unstable β-globin chain variant
- β-thalassemia trait + triplication of a-globin genes

Includes Hb variants with β^+ phenotype

Table 1.2 Secondary gene modifiers: globin genes that affect globin chain imbalance

Alpha globin chain synthesis
- Coinheritance of alpha-thalassemia (ameliorate)
- Triplication of alpha globin genes (aggravate)

HbF synthesis
- Hereditary persistence of fetal Hb synthesis (HPFH)
- XMN-1 polymorphism [CD-158(C→T](polymorphism HBG2 at position -158

Alpha-Hb-Stabilizing Protein (AHSP)

Table 1.3 Tertiary gene modifiers: genes that affect disease process but not globin chain production

Coinheritance of genes
- Hemochromatosis gene: increase iron loading (damage to tissues)
- Mutation in promoter gene of bilirubin-UDP-Glucuronyltransferase: increase in unconjugated bilirubin production (jaundice, risk of gall stones)
- Bone metabolism (COLIA1): low bone mineral density (increased risk of spontaneous fractures)
- Hereditary ovalostomatocytosis: reduce deformability of cells (rigid cells get trapped in spleen)

Attending physician's dilemma: To treat or not treat
In some countries in Asia and in Southeast Asia in the absence of guidelines, treatment is not in a defined way, ad-hoc and also follow `demand transfusion'.
Patients may be asymptomatic with Hb levels at 7 gm/dl. Studies have indicated complications do increase later in life in the absence of regular blood transfusions.

2.1 Treatment options: Practical approach

Personalized therapy remains the treatment of choice. Optimal care targets at quality of life, attainment of puberty, sexual maturation and prevention of complications. An important consideration is access to health care facilities. However, patients must have an option for best possible care at time of diagnosis to prevent complications of the disease process. Counselling, discussion on outcomes and therapeutic options keeping in mind available resources and health care facilities should be offered to all patients.

2.2 Key Points

- Clinical diagnosis: This requires careful observations for a period of time before a decision is made. Proper records are a necessity.
- Early diagnosis is a must. Patients need to be counselled as to regular follow-up for life despite being asymptomatic.

At time of diagnosis the following investigations are done:
- Complete molecular studies to identify gene modifiers
- Baseline studies informative for management: thalassemia diagnosis (Hb subtypes identified and quantified), blood group genotype, liver and renal profile, viral studies and serum ferritin.

Indications for blood transfusions

- These relate to poor quality of life, failure to thrive, growth retardation, intense erythropoiesis, skeletal deformities, progressive splenomegaly including hypersplenism and extramedullary masses.
- In special circumstances a fall of hemoglobin related to severe infection, oxidant therapy, pregnancy, heart disease, to heal leg ulcers and surgery.

3.1 Surrogate markers that indicate intense erythropoiesis: radiological measurement showing increasing expansion of the medullary cavity of the 2nd metacarpal bone, a more severe fall of Hb levels with increasing presence of nucleated red blood cells in the peripheral blood and increased levels of soluble transferrin receptor (sTfR).
3.2 The aim of blood transfusion is to suppress bone marrow activity, promote better growth and decrease iron absorption through the gastrointestinal tract. Pretransfusion Hb levels are kept between 9-10 gm/dl and iron chelation therapy commenced. Transfusions started after the age of 2 years have a risk of alloimmunisation. Success of therapy as in thalassemia major depends upon compliance to iron chelation therapy.

Complications seen in beta-thalassemia intermedia occur in the absence of blood transfusions in moderate and severe clinical phenotypes.

4.1 These are splenomegaly, bone abnormalities, extramedullary masses and leg ulcers.
4.2 In pregnancy, splenomegaly may interfere with uterine development of foetus and together with low Hb levels result in restricted intra-uterine growth of fetus (small by weight gestation). Skeletal abnormalities result in births by caesarean section.
4.3 Progressive iron overload has been associated with liver disease (liver cirrhosis and risk of hepatocellular cancer).
4.4 In advancing age, late complications cause morbidity and mortality. The Hb level is lower due to decreased erythropoietin and quality of life is affected.
4.5 Failure of commencement of therapy has resulted in splenectomy leading to increased risk of infection, hypercoagulable state, pulmonary hypertension and secondary heart failure.
4.6 The most common cause of death in TI patients with splenectomy is infection. *Splenectomy is no longer recognised as a treatment of choice.*

Stem cell transplantation

- Complications are a cause of morbidity and mortality in the absence of blood transfusions.

- Compliance to regular blood transfusions and chelation therapy can be difficult.

5.1 Hemopoietic stem cells sourced from HLA matched sibling donor (bone marrow or umbilical cord) is a modality of therapy that has resulted in cure of the disease in moderate and severe TI.
5.2 Transplantation related mortality being about 5%.

Supplements

- Globin chain imbalance and excess free labile iron result in the formation of free radicals that damage tissues.
- Zinc deficiency is seen.
- Folic acid requirements are increased as a result of ineffective erythropoiesis and chronic hemolytic anemia.

6.1 Supplements: vitamin E, chelated zinc and folic acid daily are given to patients with TI to correct these deficiencies.
6.2 Green tea a minimum of two cups a day aids the inhibition of iron absorption via the gastrointestinal tract.

Summary

- Beta-thalassemia intermedia (TI) is a clinical definition. Clinical phenotype is between thalassemia minor and major.
- Diagnosis requires careful observations over a period of time. Patients require regular follow up for life.
- Hb level and identification of gene modifiers are arbitrary guidelines as to possible clinical phenotype.
- Patients with Hb < 7 gm/dl are destined to be short (below 3rd centile on the growth chart), have splenomegaly and skeletal abnormalities. Late complications are features with advancing age.
- Splenectomy is not a treatment choice.
- Patients with Hb <7 gm/dl and moderate/severe TI with access to good quality care facilities should have regular blood transfusions and iron chelation therapy or stem cell transplantation.
- Supplements in treatment include vitamin E, chelated zinc, folic acid and drinking of green tea.

α-THALASSAEMIAS -HBH TYPE

Vip Viprakasit

Department of Pediatrics and Thalassaemia Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Clinical phenotypes associated with α thalassemia result from mutations involving the α globin gene cluster on the telomeric region of the short arm of chromosome 16 (16p 13.3). From two copies of the α globin gene per haploid genome (αα/αα), the α^0 thalassemia (when gene expression is completely abolished), is caused by lost of the both linked α globin genes (—/αα) resulting in mild hypochromic, microcytic anemia in heterozygotes. In α^+ thalassemia, the globin expression is reduced and mainly casued by single α gene deletions (-α) or mutations in one or a few nucleotides in critical regions of the α genes ($\alpha^T\alpha$). Compound heterozygotes for α^0 and α^+ thalassemia results in a severe imbalance in globin chain synthesis giving rise to excess β globin chains precipitate and form a characteristic, Hemoglobin H (Hb H) due to β globin tetramer (β_4). Patients with this syndrome have mild to moderate chronic hemolytic anemia and some might be more severe due to additional deleterious effects on terminal erythroid differentiation and red cell metabolism in particular cases with α^T mutations. Some might require regular transfusion, however nearly all of them responded well with splenectomy. It is possible that several genetic modifiers might play role on different clinical heterogeneity found in Hb H disease including co-inheritance of β thalassemia, mutations of iron-regulating genes, red cell membrane microskeletal proteins, bilirubin metabolism and erythroid specific transcription factors such as KLF-1 etc. Beside these genetic polymorphisms, variation in clinical severity in Hb H patients could also be contributed by several environmental factors from perinatal and neonatal stress, chronic infection causing active splenic function to organism that directly attacked erythroid progenitor cells such as Parvo-virus B19. Altogether, Hb H disease might not necessarily benign as usually thought and a careful diagnosis by molecular testing of globin defects and others with closely monitoring and follow up in any given patients must be obligatory.

SICKLE CELL DISEASE

Miguel R. Abboud

Department of Pediatrics, American University of Beirut Medical Center, Children's Cancer Center of Lebanon, Beirut, Lebanon

Sickle cell disease (SCD) is the most common monogenetic disorder worldwide. It is characterized by the polymerization of haemoglobin S (HbS), which results in distorted rigid red blood cells with concomitant microvascular occlusion and chronic haemolysis. Neonatal screening and modern therapy (treatment in comprehensive centres, hydroxyurea, increased use of transfusions and chelation) has led to a decrease in morbidity and mortality in children with SCD. The complications associated with SCD, however, remain a significant factor in these patients. Pulmonary complications and strokes have emerged as important causes of morbidity and mortality in patients with SCD. in adults. Management of the disease is primarily aimed at preventing and managing complications. A proactive approach utilizing several therapeutic options including haemopoietic stem cell transplantation (HSCT) in eligible patients, blood transfusion therapy, and hydroxyurea has been demonstrated to be effective in decreasing stroke rates as well as other complications and prolonging survival in patients with sickle cell disease. Allogeneic HSCT is the only potentially curative treatment, and it has a high success rate in a limited number of patients with an HLA-related donor. Transfusion therapy and hydroxyurea on the other hand are applicable to a larger number of patients. As a result of the STOP trials, blood transfusion therapy, aimed at increasing total Hb while keeping HbS below 30%, is considered the standard to prevent primary and secondary stroke in children. However, because SCD patients receiving chronic transfusions are at risk of iron overload complications, the concomitant use of iron chelation therapy is indicated. Phlebotomy, another iron-reduc-

tive treatment, has also been used in clinical practice. Hydroxyurea, which has multiple effects, including increased fetal Hb production, has been used in adults and children with SCD to prevent painful crises and acute chest syndrome and to reduce hospitalization rates.While utilization of hydroxyurea is associated with improved survival its effect on stroke prevention and pulmonary hypertension is not clear. Recently, the SWiTCH trial compared these experimental therapies (phlebotomy and hydroxyurea) with standard therapy (transfusions plus iron chelation therapy with deferasirox) for the prevention of secondary stroke and the management of iron overload in SCD patients. While no strokes were reported in those patients receiving standard, transfusion, therapy, 7 cases of stroke occurred in the cohort receiving hydroxyurea. This led to the early termination of the trial. Moreover, with regard to the control of iron accumulation, the study concluded that phlebotomy was not as effective as deferasirox in decreasing iron overload Furthermore, five-year efficacy and safety data from clinical trials of deferasirox in SCD patients were indicate that iron chelation therapy can be easily managed serum ferritin levels decreased over the long term without evidence of renal toxicity. Future research should focus on better identifying patients at risk for complications and institution of preventive therapy.

A MULTI-DISCIPLINARY APPROACH: THE VALUE OF CENTRES OF EXPERTISE IN THE CARE OF THE THALASSAEMIAS

Michael Angastiniotis

Medical Advisor to the Thalassaemia International Federation

Thalassaemias and other chronic anaemias over time become increasingly multi-organ diseases. This is the result of organ damage due to chronic anaemia and iron overload which is the result of regular blood transfusions and absorption of iron from the gut. Many complications are possible from the disease and from the necessary treatment. These include extramedullary masses which have pressure effects especially on the spinal cord, hypersplenism, severe infections, hypercoagulabilty, serious transfusion reactions, heart complications, endocrine complications, hepatic complications, bone disease and others. In addition these are chronic conditions which require often psychosocial support. All this requires the coordinated contribution of several medical specialities. The effectiveness of such a collaborative approach is best provided in expert or reference centres with experience in diagnosis and proactive planned care.

GENE THERAPY OF HUMAN BETA-THALASSEMIAS

Philippe Leboulch[1,2,3] and the LentiGlobin clinical trial study group

[1]*CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI), Fontenay-aux-Roses, France;* [2]*Inserm U962 and University Paris 11, CEA-iMETI, Fontenay-aux-Roses, France;* [3]*Genetics Division, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA*

Gene therapy of the beta-thalassaemias by transfer of a functional globin gene to hematopoietic stem cells followed by its regulated expression in the red blood cell lineage is being investigated in human patients. Compound β^E/β^0-thalassemia is the most common form of severe thalassemia in southeast Asian countries and their diasporas, and approximately half of β^E/β^0-thalassemia patients are transfusion-dependent. The only available curative therapy is allogeneic hematopoietic stem cell transplantation, although most patients do not have an HLA-matched, geno-identical donor, and those who do still risk rejection or graft-versus-host disease. Here we show that, more than 4 years after lentiviral β-globin gene transfer (transplantation performed on June 7, 2007), an adult patient with severe β^E/β^0-thalassemia dependent on monthly transfusions since early childhood has become transfusion independent for 3 years (last transfusion on June 6, 2008). Blood hemoglobin is stable around 9 g dl−1, of which one-third contains vector-encoded β-globin. This blood hemoglobin levels have been maintained in spite of the fact that monthly phlebotomies are now being performed in an effort to remove accumulated iron originated from many years of red blood cell transfusions. Part of the therapeutic benefit results from a partially dominant (below 10%), myeloid-biased cell clone, in which the integrated vector causes transcriptional activation of *HMGA2* in erythroid cells with further increased expression of a truncated *HMGA2* mRNA insensitive to degradation by let-7 microRNAs. The clonal dominance that accompanies therapeutic efficacy may be coincidental and stochastic or result from a hitherto benign cell expansion caused by dysregulation of the *HMGA2* gene in stem/progenitor cells. Comparison of genomewide integration pattern and globin gene expression in somatic hematopoietic stem cell *vs*. iPS routes in the same patient and with the same vector will also be presented. Extension of the ongoing trial and complementary or alternative approaches to increase safety and efficacy will be discussed. A second patient with severe, transfusion-dependent β^E/β^0-thalassemia was transplanted in December 2011 following the same gene therapy protocol. Early results will be presented.

HOW CAN WE IMPROVE THE OUTCOME OF HAEMATOPIETIC STEM CELL TRANSPLANT IN SEVERE THALASSAEMIA?

Suardej Hongeng

Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Hematopoietic stem cell transplant (HSCT) is the only curative therapy for severe thalassemia. However, some issues still remain unsolved problem to yield a better outcome such as unrelated donor transplant, older age patients, graft rejection and long term toxicities. I will summarize the worldwide reports of HSCT in thalassemia and will demonstrate our result of HSCT in thalassemia including related and unrelated donor transplants, reduced

intensity transplant and etc. I will also explain some special issues in HSCT in thalassemia such as how to manage older age patients to achieve a better outcome, how to deal with mixed chimerism setting to prevent further graft loss, and long term toxicities awareness.

HEMOGLOBIN F ENHANCERS: NOVEL AGENTS, NEW POTENTIAL FOR TREATMENT OF BETA THALASSAEMIAS

Suthat Fucharoen,[1] Nattawara Chaeiam,[1]
Susan P. Perrine[2]

[1]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhonpathom, Thailand; [2]Hemoglobinopathy-Thalassemia Research Unit, Department of Medicine, Pediatrics, Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA

The beta-thalassaemias are serious genetic blood diseases caused by deficiency of the beta globin chain of adult hemoglobin A and have been designated by WHO as a global health burden, due to morbidity and early mortality from its complications. An alternative approach is to pharmacologically reactivate another endogenous type of fetal or γ-globin, which is normally suppressed in infancy. Several prior generation therapeutic agents have induced γ-globin expression in beta thalassemia patients and raised hemoglobin levels, eliminating transfusion needs, and demonstrating proof-of-concept. Butyrate treatment eliminated transfusion requirements in formerly transfusion-dependent patients with treatment given for up to 7 years. However, the prior generation therapies were not applicable for widespread use, due to IV or subcutaneous administration. Currently, a novel oral dual-action therapeutic, sodium 2,2-dimethylbutyrate, entered clinical trials with encouraging effects in beta thalassemia and sickle cell disease, an oral decitabine formulation is under development, and agents with complimentary mechanisms of action can be combined for additive effects. Identification of 3 major genetic trait loci (QTL) that modulate clinical severity provides avenues for developing tailored treatments. These refinements offer renewed potential to apply fetal globin enhancers as treatment in patient-friendly regimens that can be used world-wide for these "first molecular diseases".

GENERATION OF A GENOMIC REPORTER ASSAY SYSTEM FOR ANALYSIS OF γ- AND β-GLOBIN GENE REGULATION

Kasey S.K. Chan,[1,2] Jian Xu,[3] Hady Wardan,[1]
Bradley McColl, Stuart Orkin[3] and Jim Vadolas[1]

[1]Cell and Gene Therapy Research Group, Murdoch Childrens Research Institute, Royal; Children's Hospital, Flemington Road, Parkville, Melbourne, Australia; [2]Departments of Paediatrics and Pathology, University of Melbourne, Victoria, Australia; [3]Division of Hematology/Oncology, Children's Hospital Boston, and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

A greater understanding of the regulatory mechanisms that govern γ-globin expression in humans, especially the switching from γ to β-globin which occurs immediately after birth, would help to identify new therapeutic targets for β-hemoglobinopathy patients. In order to further elucidate the mechanisms involved in γ-globin expression, a novel fluorescent-based cellular reporter assay system was developed. Using homologous recombination, two reporter genes, DsRed and EGFP, were inserted onto a 183 kb intact human β-globin locus under the control of ^{G}γ or ^{A}γ-globin promoter and β-globin promoter, respectively. The modified constructs were stably transfected into adult murine erythroleukaemic (MEL) cells and human embryonic/fetal erythroleukemic (K562) cells, allowing for rapid and simultaneous analysis of fetal and adult globin gene expression according to their developmental stage-specific expression. To demonstrate the utility of this system, we performed RNA interference (RNAi)-mediated knockdown of BCL11A in the presence or absence of known fetal hemoglobin inducers and demonstrate functional derepression of a γ-globin-linked reporter in an adult erythroid environment. Our results demonstrate that the cellular assay system represents a promising approach to perform genetic and functional genomic studies to identify and evaluate key factors associated with γ-globin gene suppression.

IMPORTANCE OF DRUG QUALITY: IMPACT ON CLINICAL OUTCOMES AROUND THE WORLD

Carlo Nalin

Novartis Pharmaceuticals Corp.

Abstract. Genuine generic drugs provide a vital and cost-effective way to meet the pharmaceutical needs of both the developing and developed world. Legitimate generic medicines are those that have met accepted standards of therapeutic equivalence, typically through bioequivalence evaluation and rigorous identity and quality testing, as verified by a stringent regulatory evaluation process. It is important to distinguish between true generic drugs and inferior copy drugs or 'substandard medicines'. Substandard medicines are defined by the World Health Organization (WHO) as "products whose composition and ingredients do not meet the correct scientific specifications and which are consequently ineffective and often dangerous to the patient" *(World Health Organization. Fact sheet N° 275. November 2003)*. Although many substandard medicines are deliberate 'counterfeit' copies produced by illegal manufacturers, they also comprise products from legitimate manufacturers who inadvertently produce copies that do not meet the necessary standards of quality and performance associated with the reference product. Substandard medicines of this nature can arise due to the lack of scientific expertise, problems with the manufacturing processes or with the quality and testing systems available *(Hudson Institute: Center for Science in Public Policy, March 2008; Caudron J-M et al. Trop Med Int Health 2009;13:1062-1072)*. The negative consequences of substandard drugs are serious and contribute to significant mortality and morbidity. Specific problems can include treatment failure, toxicity or promotion of drug

resistance. As a result, pharmacovigilance based on drug source is needed to ensure that patient safety and drug integrity is maintained.

MRI-BASED TECHNOLOGY FOR DEVELOPING COUNTRIES

John Wood

USA

Iron overload is a pressing clinical problem in tropical countries where hemoglobinopathies are extremely common. MRI has emerged as the single-most powerful tool for monitoring iron overload because it can accurately track iron deposition in any organ. However, using MRI for this purpose is challenging in developing countries because of lack of scanner and pulse-sequence availability, affordable post-processing software, and dedicated personnel to acquire and analyze the images. This talk focuses on several strategies to lower magnet demand, shorten scan time, use alternative pulse sequences, and incorporate low-cost solutions to image analysis.

MRI-BASED MONITORING TOOLS FOR IRON CHELATION

Pairash Saiviroonporn

Thailand

Magnetic Resonance Imaging (MRI) -based Iron measurement is recently gaining more acceptance as a clinical tool for monitoring iron concentration in Thalassemia patients. The measurement can be conveniently performed on various organs, such as heart, liver or pancreas. Technically, the measurement can be separated into Magnetic Resonance (MR) acquisition and post-processing-analysis processes. Robust image quality can be obtained using multi-echo gradient echo imaging in a single breath-hold time utilizing either bright- or black-blood contrast. The bright-blood technique is an earlier proven technique that is still employed with some previous MR scanner platforms. Black-blood techniques, on the other hand, are generally considered superior because flow and motion artifact suppression improves edge detection and lowers T2* variability. In post-processing analysis of the T2* measurement, however, greater heterogeneity is found but it still can provide reliable results if cares had been taken. Currently cardiac and liver iron measurements can be easily acquired in one MRI setting which can be done in less than half an hour. After the acquisition, the cardiac images were analyzed to provide quantitative result as T2* value while the liver images were also analyzed to obtain liver iron concentration (LIC) using tabulate data calculated from the measured T2* correlated to the liver biopsy result. The results from both heart and liver are utilized by clinicians to monitor the efficacy of iron chelation which are different among organs as shown in Figure 1 and 2. As a result, the MRI-based iron measurement has, therefore, become the de-facto standard for monitoring iron overload in clinical trials of the iron chelation therapy and also in clinical care of the long-term transfusion patients, especially in Thalassemia patients.

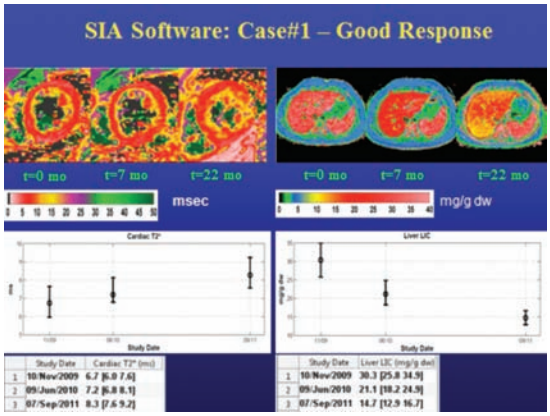

Figure 1. The iron monitoring of the heart and liver from a Thalassemia major patient presented with good responses to iron chelation.

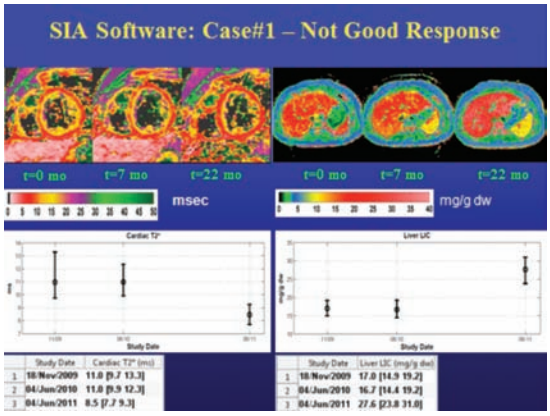

Figure 2. The iron monitoring of the heart and liver from a Thalassemia major patient presented with not good responses to iron chelation.

TRANSPLANTATION IN LOW RESOURCE COUNTRIES

Lawrence Faulkner, Naila Yaqub,[2] Sadaf Khalid, Tatheer Zhara, Saqib Ansari,[1] Tasneem Farzana,[1] and Tahir Shamsi[1]

Cure2Children Foundation, Florence, Italy, [1]National Institute of Blood Diseases, Karachi, Pakistan, [2]Pakistan Institute of Medical Sciences Children's Hospital, Islamabad, Pakistan

Abstract. BACKGROUND. Thalassemia major (TM) is the most common deadly genetic disorder, a major cause of chronic non-infectious morbidity and financial burden in many low and middle-income regions. In these settings few children reach adulthood because proper long-term supportive care is seldom available. Bone marrow transplantation (BMT) is the only available curative modality and it can be very successful and cost-effective for young children with low-risk features and a compatible related

donor. However, in countries where TM is most prevalent, there is a dire shortage of BMT centers. The Cure2Children Foundation has supported a feasibility study evaluating safety, efficacy and costs of developing a new BMT center in an underserved lower-middle-income country with relatively untrained professionals within a structured collaboration and knowledge-transfer program.

METHODS. A total of 24 consecutive patients who underwent BMT in Pakistan between September 2008 and August 2010 are included in this prospective analysis, 17 from an established bone marrow transplant center, the National Institute for Blood Diseases in Karachi, Pakistan and the initial 7 BMTs from a start up unit in a government civil hospital, the Pakistan Institute of Medical Sciences Children's Hospital in Islamabad. Patients were matched for age, nutritional status, growth, disease, disease status and post-BMT follow-up time. All patients had a matched-related sibling donor, were younger than 10 years of age at the time of transplantation, received the same conditioning regimen. All needy families could rely on a support program throughout the 8-month post-transplant period. The Cure2Children Foundation provided professional and financial support as well as a structured web-based data management and cooperation platform.

RESULTS. At a median follow up of 19.6 months (range 8.7 to 31.5) actuarial thalassemia-free survival is 85.6% and 85.7% and overall survival 94.1% and 85.7% in the established and start-up center respectively with no statistically significant differences. Other outcome indices like infectious complications, engraftment parameters, transplant-related complications, and post-BMT performance scores also did not differ. The median cost of matched-related transplants in the start-up center, including pre-BMT evaluation, was 11,513 USD (range 7,518 to 21,176).

CONCLUSIONS. Within structured cooperation strategies bone marrow transplantation for thalassemia major can be performed safely, effectively, and affordably even in start-up centers in lower-middle-income countries, like Pakistan, were most thalassemia patients live. This observation may have important implications to increase access to cure for thalassemia worldwide.

Introduction. THALASSEMIA DISEASE BURDEN. Thalassemia major is the most frequent deadly genetic defect worldwide and accounts for a substantial proportion of childhood mortality, morbidity and related healthcare expenses in many densely populated regions in North-Africa, Eastern Mediterranean, South-East Asia and Western Pacific where the carrier rate ranges from 3% to 40%.[1,2]

THALASSEMIA OUTCOMES IN LOWER-INCOME COUNTRIES. In spite of significant progress in supportive care, which may extend life expectancy well into adulthood,[3] most patients in low to middle-income areas, where thalassemia is most prevalent, do not survive beyond 20 years of age and the risk of blood-borne infections, primarily hepatitis C, is still substantial.[4]

BONE MARROW TRANSPLANTATION FOR THALASSEMIA: ACCESSIBILITY AND COSTS. Bone marrow transplantation (BMT) remains the only definitive cure for thalassemia major with reported thalassemia-free survival rates consistently over 80% in selected young patients with a related histocompatible donor.[5-7] BMT is also associated with improved quality of life[8,9] and is generally recommended in young low-risk patients with a matched related donor.[10] In areas were thalassemia is endemic there is a severe shortage of centers for its cure.[11,12] This is not only due to lack of financial resources, in fact BMT is less expensive compared to long-term supportive care,[13] but other factors may contribute such as lack of specific professional expertise or the perception that setting up a BMT center is highly complex and expensive.

THE CURE2CHILDREN EXPERIENCE SUPPORTING BMT FOR THALASSEMIA IN PAKISTAN. Cure2Children (C2C) is a non-profit and secular International Non-Governmental Organization founded by parents who lost their child to cancer. The mission of C2C is to promote and support the care of children with malignancies and severe blood disorders directly in developing countries. The Pakistan project originated from the passion, commitment and capacity of a Pakistani family with a thalassemic child transplanted in Italy by a team connected to C2C. The primary aim of this initiative has been to increase access to cure for children with thalassemia by capitalizing on available local expertise and facilities. The strategy relied on the prospective assessment of outcomes within a structured knowledge-transfer and support program applying common treatment protocols and standards for patient selection and management. We sought to assess if reported differences in outcome related to socio-economic conditions, are due primarily to patient selection, inconsistent or no access to appropriate drugs, lack of professional expertise, rather than to local conditions increasing the risk of infectious complications or causing poor family support and follow-up care. In addition we tested the hypothesis that within structured cooperation and assistance from third-party non-governmental organizations, like C2C, a relatively complex procedure like BMT could be set up safely and effectively with limited resources. PATIENT CHARACTERISTICS. A total of 24 consecutive patients with transfusion-dependent thalassemia transplanted in Pakistan between September 2008 and August 2010 were included in the analysis. Selection criteria included age less than 10 years, no hepatomegaly (liver <2cm from the right costal margin on physical examination), availability of an HLA-matched suitable related donor, clear understanding of the risks and benefits of transplant, informed consent, and no major infectious diseases or other conditions affecting transplant outcome. Hepatitis C positivity was not considered an exclusion criteria. Eligibility also required serum creatinine, bilirubin and transaminase less than twice normal values, normal chest x-ray and echocardiogram, normal age-appropriate performance scale and institutional commitment to sharing patient data on a daily basis and according to privacy regulations. Liver biopsy was not considered mandatory and absence of significant hepatomegaly was deemed sufficient to exclude high transplant-related risk. Thus, according to the standard Pesaro risk assignment,[5] these patients would be considered class I or II and have an expected cure rate with BMT of at least 85%.[14] Within this group, 17 patients were transplanted in an established private hospital, the National Institute of Blood Diseases in Karaci (NIBD), and 7 at a start-up unit developed by C2C at the Children's Hospital of the Pakistan Institute of Medical Sciences (PIMS), a large government hospital in Islamabad. This 2-bedded newly developed BMT service,

the "Simone Montomoli" Bone Marrow Transplant Unit, was created in a 150 square meter total space provided by the hospital administration and renovated with less than 50,000 USD, including basic equipment. Institutions signed formal agreements with the Cure2Children Foundation specifying the above selection criteria and treatment protocol. All patients were admitted in single rooms with split air conditioning, private bathrooms and daily cleaning. Positive pressure gradients or centralized HEPA filtration systems were not considered mandatory while blood product irradiation, cyclosporin blood level monitoring and cytomegalovirus (CMV) reactivation assessment, by either pp65 antigenemia or real-time PCR, were required. Hand washing was strictly enforced, however while wearing gloves and gowns this was not. The two patient groups were matched for age, growth and nutrition, diseases, disease status, graft type, conditioning, GVHD/rejection prophylaxis and post-BMT follow-up time (Table 1).

Bone marrow transplant procedure. The following conditioning regimen was utilized: Busulfan 3.5 mg/kg day in 4 divided doses on days -10 to -7 (total dose 14 mg/kg), Thiotepa 10 mg/kg/day in two divided doses on day -6 (total dose 10 mg/kg), and Cyclophosphamide 50 mg/kg day once daily on days -5 to -2 (total dose 200 mg/kg) followed by the infusion of freshly harvested HLA-compatible bone marrow on day 0. GVHD prophylaxis consisted of cyclosporin A starting at 5 mg/kg i.v. from day -2 to +5 than 3 mg/kg i.v. from day +6 to +22 to be followed by 10 mg/kg/day in two daily oral doses for up to day +90 after which it was tapered by -5%/week and discontinued at 7-8 months post-BMT unless otherwise indicated. A short methotrexate course consisting of 10 mg/msq i.v. on days +1 (24 hours after marrow infusion), +3 and +6, with folinic acid rescue at 24 hours after each methotrexate with 3 doses of 10 mg/msq i.v. at 8 hour intervals, i.e. at hours +24h +32h and +40h. Methylprednisolone at 0.5 mg/kg/day i.v. from day -1 to +30 and tapered by -1/3 every 5 days over 15 days and stopped on day +45 is also used.

Infection control, treatment and prophylaxis. For anti-helminthic prophylaxis mebendazole 100 mg twice daily for three days was administered before conditioning. For candida infection prevention fluconazole 3-6 mg/kg/day as a single dose was used and for herpes virus acyclovir 250-500 mg/m^2/dose three times a day, both drugs were administered from day +1 to +90. CMV reactivation was monitored weekly from day +15 to +90 post-BMT. No routine antibacterial prophylaxis was used. For antifungal therapy amphotericin B was considered the first-line drug and voriconalzole second line. For CMV activation (any antigenemia positivity or > 500 CMV DNA copies/ml) both ganciclovir and foscarnet were available. Co-trimoxazole at 5 mg/kg/dose twice daily for three consecutive days a week was administered for *Pneumocystis* prophylaxis from the day neutrophil counts reach 500/mcl to day +90.

Family support program. A patient coordinator as well as housing and monthly allowance was provided by C2C. This family support program was implemented throughout the eight months post BMT.

Collaboration methodology. Structured cooperation and communication strategies involving international and national transplant specialists were employed. Management standards for therapy administration, central venous access, severe pancytopenia, immunosuppression, and hospital infection control were addressed by local training, a specific web-based open-source data management software developed by C2C (CDATA, www.cure2children.org/ws/thdb) and Skype videoconferencing (www.skype.com). Patient-specific treatment plans were provided as Microsoft Excel-generated precalculated treatment sheets meant to reduce the physician's workload and prescription errors and also to supply nurses with clearly written order sheets in keeping with good clinical practices.

Statistical analysis. Statistical analysis was perfomed using GraphPad Prism software version 5 March 2007 (www.graphpad.com/prism/Prism.htm). Kaplan-Meier survival curves were compared by Log-rank (Mantel-Cox) Test and patient characteristics by Mann-Whitney nonparametric test using two-tailed p values. Anthropometric z-score values were calculated with the World Health Organization shareware Anthro.[15] For patients above 60 months of age WHO Child Growth Standards reference tables were used.[16]

Table 1. Characteristics of patients treated at the established institution (NIBD) versus a start up service (PIMS).

	Established unit	Start-up unit	*p value*
Age (years)	3.5 (1.8 to 9.4)	3.0 (0.9 to 5.4)	0.43
Gender	8 M, 9 F	6 M, 1F	
Height for age (z-score)	-0.82 (-3.47 to 1.92)	- 1.17 (-2.48 to 0.72)	0.94
Weight for age (z-score)	-0.51 (-3.55 to 0.73)	-0.72 (-2.60 to 0.12)	0.43
Body mass index (z-score)	-0.11 (-2.78 to 1.68)	-0.71 (-2.49 to 0.77)	0.53
Pre-BMT Ferritin	1200 (289 to 2960)	2476 (616 to 8008)	0.58
Consanguinity	14/17 (82%)	5/7 (71%)	
Donor thalassemia minor	10/17 (59%)	6/7 (86%)	
Nucleated cell dose/kg×10^8	8.6 (1.4 to 23)	5.8 (3.1 to 8.0)	0.27
Follow up from BMT (months)	20 (10.9 to 31.5)	17.7 (8.7 to 24.2)	0.59

ENGRAFTMENT DATA. At a median follow up of 20 months (range 10.9 to 31.5) and 17.7 (range 8.7 to 24.2) for patients managed in an established unit or in a start-up unit respectively, no significant difference in outcomes was observed with actuarial thalassemia-free survival of 85.6% and 85.7% (log rank p value 0.8609) and overall survival 94.1% and 85.7% (log rank p value 0.5322) respectively. (Table 2)

ADVERSE EVENTS. (Table 3)

COST ANALYSIS. (Table 4)

Discussion. To our knowledge this is the first study prospectively comparing homogeneous patient groups undergoing bone marrow transplantation in start-up versus established BMT centers in low-resource settings in the context of a structured knowledge-transfer strategy. The objective of this study, in keeping with the mission of the Cure2Children Foundation, was to assess the potential to extend access to BMT for severe hematological disorders in underserved regions. There is general perception that bone marrow transplant centers need complex engineering standards requiring undue investments, even more in poor countries were infectious risk may be higher. In fact there is no evidence that the latter is true.[17-19] Even if available international guidelines call for placement of allogeneic BMT recipients in highly protected environments with positive pressure gradients, intensive air exchange and filtration,[20] these recommendations are not based on well-designed clinical trials. In fact, safe care of allogeneic transplantation recipients has been reported even in the outpatient setting.[21,22] Many widely held practices which may unnecessarily increase BMT-related expenses are being reconsidered worldwide and cost-containment considerations are even more important in settings with more limited resources. In this study patients and their caretakers were admitted to single rooms with private bathrooms and split air conditioning. Strict hand washing of all personnel and visitors was enforced and BMT units were cleaned daily. Infectious complications observed in Pakistani centers did not seem to be substantially different from those encountered in Europe or North America, with the exception of two possible cases of probable pulmonary tuberculosis. CMV reactivation was the main opportunistic infection observed. Of the two centers participating in this project one was a newly developed service heavily relying on initial local training followed by daily interaction via video-conferencing and web-based data management, the second an established BMT service which had been performing transplantation for 8 years. This experience may set a useful precedent to extend access to bone marrow transplantation in lower-middle income regions and limit the emigration of thalassemia major patient to richer countries or even increase attraction of patients from abroad. In our experience BMT outcomes in low-risk cases with

Table 2.

	Established unit	Start-up unit	*p value*
Median day ANC>500/μL	19.5 (12-35)	15 (10-23)	0.15
Median day Plt >20.000/μL	22.5 (10-49)	15.5 (12-20)	0.07
Median n. Plt transfusions	6.5 (1-26)	4 (1-18)	0.25
Median n. RBC transfusions	2 (1-6)	2 (0-7)	0.47
Last RBC transfusion day	12.5 (1-36)	16 (0-152)	0.59
Median discharge day post-BMT	26 (12-60)	38.5 (22-109)	0.02

Table 3. Summary of adverse events.

	Established unit	Start-up unit
Infections		
CMV activation	7 (41%)	3 (43%)
Aspergillosis (probable or proven)	0	0
Fever and neutropenia	11 (65%)	4 (67%)
Tuberculosis (probable)	1 (6%)	1 (14%)
Acute GVHD grade 1-2	1 (6%)	2 (29%)
Acute GVHD grade 3-4	0	2 (29%)
Limited chronic GVHD	1 (6%)	1 (14%)
Extensive chronic GVHD	0	0
Hemorrhagic cystitis	2 (17%)	1 (14%)
Mucositis (> ECOG grade 1)	5 (29%)	2 (28%)
Death	1 (6%)	1 (14%)

Table 4. Cost comparison. Pakistan costs obtained from the seven transplants performed in the start-up unit in a government hospital, all patients had at least 8 months follow up post-BMT.

	Start-up unit Median (range)
Pre-Transplant evaluation	$ 532 (141-1,026)
Diagnostic	$ 1,704 (733-4,548)
Blood products	$ 132 (88-374)
Hospitalization charges	$ 535 (455-1,320)
Surgical devices (CVL)	$ 396 (311-437)
Drugs	$ 1,577 (1,212-5,518)
Professional Cost	$ 4,324 (3,817-10,660)
Follow up cost	$ 1,714 (375-2,350)
Total	$ 11,513 (7,518-21,176)

a compatible related donor were comparable to those obtained in high-income countries but with one-tenth of the costs. Given the great case load, high success rates and much lower costs, thalassemia BMT programs in developing countries may achieve high standards of care, international competiveness and self-sustainability. This may not only contribute to boost advances in the cure of thalassemia worldwide but also to increase access to BMT for other life-threatening diseases such as severe aplastic anemia and high-risk leukemia, and contribute to biomedical research. The prospect of cure may also increase the attraction of affected families and increase opportunities to cascade screening and prevention programs[23] as well as improve compliance with supportive care. The lower than expected incidence of severe acute and chronic GVHD in Pakistani patients might be attributable to consanguinity resulting in reduced minor HLA disparities as well as on effects due to ethnicity.[24] This study, in addition to the establishment of a proof of principle with potential important effects on increasing the number of BMT centers and empowerment of tertiary medical care in developing countries, might also be relevant to health care cost containment, comparative-effectiveness assessments, and current practices of bone marrow and stem cell transplantation worldwide.

Acknowledgments. This work was possible thanks to the support of Fondazione Monte dei Paschi di Siena, Italy.

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1st Pan-Asian Conference on Haemoglobinopathies

8-10 February 2012, Bangkok - Thailand

POSTERS

SPLENECTOMY OUTCOMEIN HB E/B THALASSAEMIA PATIENTS A SINGLE CENTRE EXPERIENCE FROM INDIA

Prakas Kumar Mandal,* Malay Kumar Ghosh,** Sanjay Misra,*** Tufan Kanti Dolui,**** Maitreyee Bhattacharrya*****

*Post doctoral trainee (clinical Hematology); **Professor & Head; ***Biochemist; ****Asstt. Professor; *****Professor Department of Hematology, Nilratan Sircar Medical College, Kolkata, INDIA

Introduction. Hb E/b thalassaemia by far the commonest form of thalassemia intermedia (TI), affects millions of people in the world. It is an important health problem in the Indian subcontinent and Southeast Asia. Its phenotype ranges from mild anemia to severe transfusion-dependent thalassemia major necessitating splenectomy in many patients.
Methods. In this study, total of 41 cases of splenectomised Hb E/b thalassaemia patients were studied retrospectively to evaluate splenectomy outcome. A detail history regarding age of splenectomy, transfusion requirement before and after splenctomy, whether on prophylactic antibacterial and anti malarials, febrile episodes requiring hospitalization, history of malaria were taken from the patients . A thorough physical examination was done for facial deformities, pubertal growth. Baseline Hb% before and after splenectomy, evidence of pulmonary hypertension, extramedullary hematopoiesis noted from patients record.
Main theme. HemoglobinE –beta thalassaemia patients often present as thalassaemia intermedia and many of them required splenectomy. Splenectomy though helps in reducing transfusion requirements and growth, it has the disadvantages of increasing the susceptibility of infections including malaria. The study was done to evaluate the course of disease and various complications following splenectomy in HbE-beta thalassaemia patients leaving in a developing country like India.
Results. Total no of registered HbE-beta thalassaemia patients is 1240. Out of these 41 (3.3%) underwent splenectomy. , Seven (17.07%) of these were diagnosed before the age of 2 years, 15 (36.57%) between 2-10 years and 14 (34.1%) after 20 years. Thirteen (31.7%) underwent splenectomy before 10 years , 19 (46.3%) between10-20 years and 9 (21.96%) after 20 years of age. Indication for splenectomy was huge splenomegaly leading to mechanical discomfort in 19 (46.3%) and increased transfusion requirement in the rest. Twenty six (63.4%) patients had base line hemoglobin < 6gm% and the rest 15 (36.5%) between 6-7.3g% prior to splenectomy. Only 3 (7.3%) patients had baseline Hb% <6g% after splenectomy. In 25 (60.9%) patients transfusion requirements reduced by more than 10 units /year but in 6 (14.6%) transfusion requirements per year was 10 units more after 5 years of splenectomy. Twenty seven (65.8%) had facial deformities and 22 (53.6%) did not have pubertal growth. Two (4.8%) developed pulmonary arterial hypertension and 4 (9.7%) had evidence of extramedullary hematopoiesis. None of the patients were on prophylactic antibiotics or antimalarials. Fourteen (34.1%) required hospitalization for fever and 6 (14.6%) suffered from malaria.
Conclusion. Splenectomy though reduces requirement of blood transfusion it perhaps does not prevent skeletal abnormalities and delayed puberty. In a country like India patients should be on antibacterial and antimalarial prophylaxis after splenectomy.

GROWTH STATUS IN β-THALASSEMIA MAJOR: EXPERIENCE OF A TERTIARY CARE CENTRE IN INDIA

Prasanna Kumar K.,[1] Lincy Mathew,[2] Anand Prakash,[3] Fulton D'Souza,[4] Phadke K.[5]

[1]Research Assistant, Pediatrics Research Unit, St. John's Research Institute, Bangalore; [2]Post graduate student, Dept of Pediatrics, St John's Medical College Hospital, Bangalore; [3]Assistant Professor, Dept of Pediatrics, St John's Medical College Hospital, Bangalore; [4]Associate Professor, Dept of Pediatrics, St John's Medical College Hospital, Bangalore; [5]Professor and Head of Department of Pediatrics, St. John's Medical College Hospital, Bangalore

Introduction. Growth failure in β-thalassemia major has been recognized for many years, and has persisted despite major therapeutic advances. The increasing mean survival of these children urges the need for appropriate intervention strategies to aid in the improvement of growth status.
Objective. To assess the growth status of children with β-thalassemia major and to determine the predictors of growth status in these children.
Methods. A prospective study conducted over 12 mths duration in children with β-thalassemia major at a tertiary care centre, St. John's Medical College Hospital, Bangalore. Results were analysed using SPSS software, version 17.0 for Windows.
Results. Sixty-one children were included in the analysis. Mean age was 8.0±3.7yrs; 57% males. Mean age at diagnosis was 5mths. Pubertal changes were seen in 3 boys and 7 girls. One girl child had attained menarche.
GROWTH STATUS. All subjects showed growth retardation with advancing age. In children below 9yrs, 17% were stunted and 60% were underweight. In children after 9yrs of age, 58% were stunted and 92% were underweight. Between genders, growth retardation was more pronounced

after the age of 9 yrs in boys and 12 yrs in girls. Follow up data revealed, poor increments in growth. Height velocity in children <9yrs was 3.3±1.6cm/yr; >9yrs was 0.5±0.4 cm/yr, p=<0.001. Moreover, height velocity in children with pre-transfusion Hb>9gm/dL was 3.9±2.4cm/yr and those with Hb<9gm/dL was1.1±0.3cm/yr, p=0.004. Univariate analysis: Height velocity showed significant correlation with pre-transfusion Hb (r 0.506, p <0.001), volume of transfusion (r 0.526, p <0.001) and serum ferritin (r -0.475, p <0.001). Multivariate analysis: Pre-transfusion Hb (R 0.881, R^2 0.776, p <0.001) emerged to be the single best predictor of height velocity.

Conclusion. This study has shown that children with β-thalassemia major experienced growth retardation above 9 yrs of age with pre-transfusion hemoglobin being the single best predictor of growth. Hence, good maintenance of pre-transfusion hemoglobin could improve the growth status in these children.

SPLENECTOMY IN β-THALASSAEMIA MAJOR: INDIAN EXPERIENCE AT A TERTIARY CARE CENTRE

Prasanna Kumar K.,[1] Purushotham V.R.,[2] Anand P.,[3] Fulton D'Souza[4]

[1]Research Assistant, Pediatrics Research Unit, St. John's Research Institute, Bangalore; [2]Post graduate student, Dept of Pediatrics, St.John's Medical College Hospital, Bangalore; [3]Assistant Professor, Dept of Pediatrics, St.John's Medical College Hospital, Bangalore; [4]Associate Professor, Dept of Pediatrics, St.John's Medical College Hospital, Bangalore.

Background. Long term management of children with β-thalassaemia major lies in maintaining good pre-transfusion hemoglobin and minimizing the iron overload status. Splenectomy is often performed in an attempt to decrease transfusion requirements and overcome hypersplenism. The timing, benefits and risks of splenectomy remain questionable.

Objective. To perform an audit of the β-thalassaemia major children who underwent splenectomy in our centre. We studied the hemoglobin, blood transfusion patterns and iron status prior to splenectomy and compared the change in these parameters post-splenectomy. The validity of indications for splenectomy and the incidence of post-splenectomy infections were also studied.

Methods. A cross-sectional study conducted in splenectomised children with β-thalassaemia major at a tertiary care centre, St. John's Medical College Hospital, Bangalore, Southern India. A semi-structured questionnaire was administered for the purpose of data collection. Results were analysed using SPSS software, version 17.0 for Windows.

Results. A total of 185 children with β-thalassaemia major have been registered in our Thalassaemia Clinic, out of which 126 children continue to visit our centre at regular intervals for medical care and blood transfusions. All children were categorized into different age groups: <1 yr (n=5), 1 to5.0 yrs (n=27), 5.1 to 10.0 yrs (n=38), 10.1 to 15.0yrs (n=36) and >15yrs (n=20). Splenectomised children included 22% (28/126) with a mean age of 13.1±4.7 (range, 7-21) yrs. Among the 28 splenectomised children the mean age at 1st blood transfusion was 6.7±3.8mths. The mean age at the time of splenectomy was 10.2±3.3yrs with a post-splenectomy follow up duration being 3.1±2.2 (range, 0.5-8) yrs. Indications for splenectomy included increased blood transfusion requirement, hypersplenism, large spleen and physical discomfort. The number of blood transfusions (BT) reduced post splenectomy (pre:16.0 BT/yr to post:11.3 BT/yr, p<0.001). The total amount of BT received also reduced to two third (pre:157ml/kg/yr to post:104ml/kg/yr, p<0.001). The average pre-transfusion hemoglobin increased post-splenectomy (5.7gm/dL to 8.6gm/dL, p=0.001). The Hb drop per week decreased from a pre-splenectomy mean of 0.61 (0.27-1.04) gm drop/week to a post-splenectomy mean of 0.41 (0.25-0.70)gm drop/week, p<0.001. Serum ferritin was found to be elevated post splenectomy (pre 2490ng/mL, post 3680ng/mL; p=0.01). Majority (82%, 23/28) of the children had no postoperative complications. Of the remaining five (18%, 5/28) children, three had thrombocytosis alone and two other children had culture positive sepsis. There were no episodes of symptomatic thrombosis.

Conclusion. This study has shown that all children with β-thalassaemia major benefited from splenectomy especially in the setting of an increase in transfusion requirements seen during the early second decade. This audit has also shown that there are definite risks associated with splenectomy which are difficult to predict. Hence, splenectomy should be done only after careful consideration.

PRENATAL DIAGNOSIS FOR THALASSAEMIA – AN EXPERIENCE FROM WEST BENGAL

Sarmistha Sanyal,[*] Malay Ghosh,[**] S. Misra,[***] Tufan Kanti Dolai,[****] Maitreyee Bhattacharyya

[*]Research Officer, prenatal diagnosis of thalassaemia project; [**]Professor & Head, In-charge, nodal center, State Thalassaemia Control Programme; [***]Biochemist , Member, State Thalassaemia Control Programme; [****]Assistant Professor; [*****]Professor, Member, State Thalassaemia Control Programme Hematology Department, N.R.S Medical College, Kolkata, West Bengal, India

Introduction. Thalassaemia and other hemoglobinopathies are major public health burden in West Bengal Prenatal diagnosis (PND) is necessary along with awareness generation and carrier detection to control thalassaemia. The study was done to assess the acceptability and efficacy of prenatal diagnosis in control of thalasseamia in population of West Bengal.

Methods. Study population includes a. voluntarily turned up carrier couples, b. carrier detected during antenatal screening programme. Fetal sample for analysis was obtained by Chorionic Villus Sampling (CVS), Amniocentesis and Cordocentesis/placental biopsy depending on gestational age. Mutational screening is done by CRDB, confirmed by ARMS.

Main theme. In cases where both the couples are carrier for thalassaemia , there is 25% chances in each pregnancy to have a thalassaemia major child .Once the couples

are detected to be carrier after marriage only way to have a carrier or normal child is to go for pre-natal diagnosis. Mutations present in the couples detected first. Chorionic villous sample is analysed for presence of these mutations. Depending on the no of mutations present in the fetus, fetus is declared normal, carrier,or affected. In case the fetus is affected , couples are left with the option of terminating the pregnancy.
Results. Total 56 cases underwent PND, 3 from antenatal screening as a part of State Thalassaemia Control Programme, 32 from Thalassaemia OPD (couples planning for second pregnancy, already having a thalassaemia child under treatment in the clinic), 18 were referred by gynaecologists and 3 came voluntarily. Out of 2496 antenatal mother screened, 298 (~8.4%) were carriers. Fifteen (~9.31%) couples were carriers, Three couples were both Hb E carrier. Out of rest 12, PND could be done in 3 , 9 turned up after 20 weeks of pregnancy (75%). Out of 56 cases, 41 (73.21%) were β thal trait, 4 (7.14%) each were homozygous for beta thal & 3 (7.89%) were compound heterozygous for Hb E -β and 2 (5.26%) were normal. There was 3(7.89%) spontaneous abortion. Mutations found are IVS1-5(G-C), IVS1 -1 (G-T), F/S 8/9, Hb E and 619bp del.
Conclusions. While reliable prenatal screening & diagnosis for thalassaemia is readily available in West Bengal, it is clear that most of the couples are not benefitting from this. Awareness generation is still a primary requisite to make women register early at antenatal clinics and bring their spouses for screening when required.

THALASSEMIA AWARENESS AMONG PEDIATRICIANS IN NORTHEAST REGION OF INDONESIA

Stefanus Gunawan, Elisa Iskandar, Diana Suryanto, Juliawaty Salim, Max FJ Mantik

Department of Pediatric Prof. RD Kandou Hospital, Faculty of Medicine Sam Ratulangi University, Manado, Indonesia

Background. Northeast region of Indonesia comprised of North Sulawesi, Gorontalo and North Maluku province with population of approximately 4.5 million. However, very few cases of thalassemia were reported from hospitals in this region.
Objectives. To know the awareness of pediatricians working in northeast region of Indonesia about thalassemia.
Methods. A cross sectional survey was conducted in August 2011. Pediatricians were asked to fill a questionnaire which consisted of questions about diagnosis, treatment, prognosis and prevention of thalassemia.
Results. Forty one out of 47 questionnaires were returned, with a response rate of 87.2%. Seventy four percent of the pediatricians had sufficient knowledge about thalassemia. Among the pediatricians, 58% stated that they had suspected patients suffered from thalassemia, but only 41% asked for laboratory workup to confirm their diagnoses. When dealing with anemic children, 87% pediatricians requested complete blood count and bloodsmear, 56% analyzed red blood cell index, but only 14.6% analyzed Mentzer index and/or RDW index. Ninety percent of the pediatricians decided to refer their thalassemia patients to pediatric hematologist or to the referral hospital. Nineteen percent happened to give thalassemia education to the society. Most of the pediatricians (97%) agreed to give pre-marital counseling or thalassemia screening towards patients with thalassemia trait, but only 17% agreed to do newborn screening.
Conclusions. Thalassemia awareness among pediatricians in northeast region of Indonesia is inadequate. Educational effort should be made to improve the awareness of thalassemia among pediatricians in this region.

ALPHA AND BETA-THALASSAEMIA IN MALAYSIA: A NEED FOR MOLECULAR DIAGNOSIS

Mary Anne Tan Jin Ai, Kim-Lian Tan, Elizabeth George

Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Malaysia, Hematology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

Thalassaemia is a public health problem in Malaysia and about 4.5% of Malaysians are β-thalassaemia carriers. Co-inheritance of α-thalassemia with homozygosity or compound heterozygosity for β-thalassemia may ameliorate β-thalassemia major. A wide range of clinical phenotypes is produced depending on the number of α-thalassemia alleles (-α/αα, —/αα, —/-α). The co-inheritance of β-thalassemia with α-thalassemia with a single gene deletion (-α/αα) is usually associated with thalassemia major. In contrast, the co-inheritance of β-thalassemia with two α-genes deleted in *cis* or *trans* (—/αα or -α/-α) generally produces β-thalassemia intermedia. DNA was extracted from patients' blood using sodium-dodecyl sulphate and proteinase-K. DNA amplification to confirm the Southeast Asian (SEA) deletion was carried out using a Duplex-PCR. The $-\alpha^{3.7}$ deletion sequence was amplified as a 1.8 kb fragment and the $-\alpha^{4.2}$ deletion sequence as a 2.1 kb fragment using Gap-PCR. The Amplification Refractory Mutation System (ARMS) and genomic sequencing was used to confirm β-globin gene mutations. Alpha- and β-thalassaemia interactions were found in 12.7% (41/322) of the β -thalassaemia patients studied. The SEA deletion was the most common defect observed in the Chinese (8.5%). Out of the 41 β-thalassaemia carriers with co-inheritance of α-thalassaemia, double heterozygosity for α- and β -thalassaemia was confirmed in five couples (12.2%). The co-inheritance of α -thalassaemia with the SEA deletion is the major problem in couples with β-thalassaemia. It is necessary to carry out molecular testing to exclude α-thalassaemia in these individuals with almost normal MCV and MCH values. The α- and β-thalassaemia defects detected confirm the large heterogeneity of mutations in the multiracial Malaysian population.

CLINICAL AND MOLECULAR SPECTRUM OF BETA-THALASSAEMIA INTERMEDIA IN MYANMAR PATIENTS AT YANGON GENERAL HOSPITAL

Sein Win,[1] Rai Mra,[1] Ne Win,[2] Htun Lwin Nyein,[1] Aye Aye Gyi[1] and Moe Hein[1]

[1]*Department of Clinical Haematology, Yangon General Hospital, University of Medicine 1, Yangon;* [2]*National Health Laboratory, Yangon*

Introduction. β-thalassaemia is common in many Asian countries including Myanmar. The clinical presentations of Myanmar patients with β-thalassaemia intermedia (β-TI) are widely variable and the nature of molecular defects is heterogeneous.
Method. The clinical data of 103 Myanmar patients with β-TI including HbE/β-TI attending to the Department of Clinical Haematology, Yangon General Hospital during two-year period from August, 2008 to July, 2010 were studied. The β-thalassaemia mutation analysis of was done at the National Health Laboratory, Yangon by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique using six sets of primers known to be common in Southeast Asian reagion.
Main theme. To study the clinical features and molecular defects in Myanmar β-TI patients and to find out the phenotype/genotype correlation
Results. As identified by haemoglobin electrophoresis using isoelectric focusing, 94 patients (91.26%) were HbE/β-TI, and 9 patients (8.74%) were β-TI patients. Mean age of patients was 26.81 ± 11.41 years (range 12-66). Male to female ratio was 1:1.19. Myanmar races (Bamar, Kayin, Shan, Yakhine, and Mon) constituted 79.61% and 20.39% were either Chinese, or Indian, or mixed Myanmar - Chinese or Myanmar - Indian. Facial deformity was observed in 79.61%. Average height was 157.02 ± 9.32 cm. Average weight was 45.13 ± 7.867 kg. Mild skin pigmentation was observed in 44.7%, and marked skin pigmentation in 30.1%. Average size of liver was 4.9 ± 3.73 cm. Average size of spleen in non-splenectomised patients was 8.32 ± 5.2 cm below left costal margin. Mean age of onset of symptoms was 13.17 ± 10.41 years (range 2-55). Mean haemoglobin at diagnosis was 6.51 ± 1.68 g/dl (range 2.2-10.3g/dl). Transfusion history was seen in 91.26%. But regular transfusion (≥2/year) was observed in 45.63% only, and sporadic transfusion (≤1/year) was observed another 45.63%. Mean age at first transfusion was 13.47 ± 9.67 years. Red cell alloimmunisation was detected in 9.71%. Anti-E anti-rhesus antibody was detected in 9 patients (42.86%), anti-c anti-rhesus antibody in 7 patients (33.33%), antibodies against Kidd blood group anti-Jk^a and antiJk^b in 2 patients each (9.52%), and antibodies against Lewis blood group anti-Le^a in 1 patient (4.76%). Splenectomy had been done in 30.1%. Mean age at splenectomy was 22.1 ±13.67 years (range 4.5-62). Mean rise in hemoglobin after splenectomy was 1.67 ± 1.003 g/dl. Complications of splenectomy were observed in 16.12% of splenectomised patients. Complications of splenectomy were sepsis (16.12%), primary haemorrhage (3.22%), poor wound healing (6.44%), and operative mortality (3.22%). Past history of bone fracture was observed in 26.21%. Biliary complications (gallstone, sludge, thickened gall bladder) were observed in 28.16%. Cardiac failure was detected in 13.59%, and thrombosis in 1.94%. Regarding education, 24.27% finished high school and 30.1% were attending high school. But, 39.81% had left the school for school performance or frequent school absence or both. Delayed puberty was seen in 25.53% of male and 85.71% of female. The outcome of 35 pregnancies were full term babies 91.43% including one twin, abortions 5.71%, premature baby 2.86%, and neonatal death 2.86%. Normal serum ferritin level was observed in 18.4%. The remaining 81.6% had raised serum ferritin. Serum ferritin between 1000-2000 ng/ml was observed in 34.0%, and serum ferritin over 2000 ng/ml was observed in 17.5%. Altogether 12.61% had deranged glucose metabolism. Hepatitis B surface antigen (HBs Ag) was positive in 3.88%. Anti-Hepatitis C antibody was found to be positive in 12.62%. Molecular analysis was made for all 103 patients by ARMS-PCR using already known six common mutations of Myanmar population. Four mutations were observed in 83 cases (80.6%): IVS 1-1 (G→T) in 28.16%, CD41/42 (-TCTT) in 28.16%, CD17 (A→T) in 18.45%, and IVS 1-5 (G→C) in 5.83%. Among them, HbE/β mutation was observed in 77 cases and single β-mutation was observed in 6 cases. Among 77 cases with HbE/β mutation, 71 cases were HbE/β^0 mutation and 6 cases were HbE/β^+ mutation. Double heterozygous of β-thalassaemia intermedia was not observed in this study. The other two mutations IVSII-654 (C→T) and -28 (A→G) were not identified in this study, and beta-thalassaemia mutation was not identified in remaining 20 cases (19.4%) by above 6 sets of primers. There is no statistically significant correlation between genotype and phenotype score of patients with Hb E beta-thalassaemia intermedia.
Conclusion. The clinical presentations of Myanmar patients with β-thalassaemia intermedia (β-TI) are widely variable and the nature of molecular defects is heterogeneous. Most Myanmar β–TI patients were HbE/β-TI (91.26%). Regular transfusion was not needed in 54.37% with mean haemoglobin at diagnosis 6.51g/dl. Four β-thalassaemia mutations were observed in 80.58% of Myanmar thalassaemia patients and may be useful for screening. There is no genotype and phenotype correlation in patients with Myanmar β–TI patients.

THE PREVALENCE OF HAEMOGLOBINOPATHY AMONG ADOLESCENTS OF PYIN OO LWIN TOWNSHIP

Tin Tin Thein,[1] Aung Kyaw Kyaw,[1] Saw Myat Thwe,[1] Zaw Win Tun,[1] Khin Moe Aung,[1] Aung Tun,[2] Aye Aye Thaw,[3] Ne Win[4]

[1]*Department of Medical Research (Upper Myanmar);* [2]*School and Youth Health Project, Department of Health;* [3]*National Nutrition Center, Department of Health;* [4]*National Health Laboratory, Department of Health*

A field and laboratory based cross-sectional analytical study was conducted to assess the prevalence of haemoglobinopathy in Shan ethnic group, Myanmar and determined the iron deficiency as main cause of anemia. A total of 155 high school students in Upper Myanmar were selected by simple random sampling procedure. The prevalence of haemoglobinopathy in Shan ethnic adolescent was 27 (17.4%), out of which beta thalassaemia trait and alpha thalassaemia trait were 1 (0.6%) each, haemo-

globin E trait was 23 (14.8%) and haemoglobin E beta thalassaemia was 2 (1.3%). This study could detect anemia in 41 students (26.5%), out of which 15 (36.6%) were associated with iron deficiency, 10 (24.4%) were related with haemoglobinopathy and 16 (39%) cases were due to other causes. Although the sample size was not large enough, the findings strongly indicate that anaemia was considerably common in adolescents where iron deficiency was the causal factor in half of the adolescent anaemia. The haemoglobin level was statistically significant between the normal and haemoglobinopathy cases ($p<0.02$) in this study. The prevalence of anaemia is still high and the common causes are iron deficiency anaemia and haemoglobinopathy. For that reason, there is a need to do the screening of the haemoglobinopathy and iron deficiency to prevent the thalassaemia and their complications and to assist the Prevention Program of Thalassaemia.

TO DETERMINE THE AWARENESS REGARDING ANTENATAL TEST AMONG PARENTS OF THALASSAEMIA MAJOR PATIENTS WITH MORE THAN ONE AFFECTED CHILDREN

Samina T. Amanat, Saima Ehsan, Huma Abdul Shakoor

P.A.E.C General Hospital H/11-4 Islamabad

Method. This cross-sectional study was carried out from May 2009 to December 2009. Parents of thalassemia major patients with more than one affected children presenting in O.P.D. of Jamila Sultana Foundation were interviewed regarding reasons for having second thalassemia major child on a pre-designed proforma.
Result. A total of 95 parents were interviewd. Majority of mothers were illiterate whereas only 25% of fathers had college education. 65% of the parents claimed lack of awareness of availability of any antenatal diagnostic facility as major reason for having second thalassemic child.30% cited financial constraints and only 5% had religious reasons for not availing ante natal diagnostic facilities.
Conclusion. Study showed still majority of parents were unaware of existence of antenatal diagnois of β-thalassemia major. Illiteracy, poverty and ignorance are the major factors. Religious beliefs played a minor role. Therefore, comprehensive preventive programmes are required with grass root implementation for prevention of β thalassemia.

THALASSAEMIA ERADICATION PROGRAMME: AN EVALUATION OF THE SRI LANKAN EXPERINCES IN MASS SCREENING

Perera H.A.A.P.K.,[1] Dayananda Bandara W.,[2] Mudiyanse, R.M.[3]

[1]Medical officer in charge, National Thalassaemia Center, Teaching hospital, Kurunegala, Sri Lanka and MSc. Medical Administration - trainee, Post Graduate Institute of Medicine, University of Colombo, Sri Lanka; [2]Consultant Pediatrician in charge, National Thalassaemia Center, Teaching hospital, Kurunegala, Sri Lanka; [3]Senior Lecturer and Consultant Pediatrician, Faculty of Medicine, University of Peradeniya, Kandy, Sri Lanka

Introduction
Thalassaemia is an inherited incurable disease of blood, which is genetically transmitted from parents to their offspring. As quoted, "management of Thalassaemia is very expensive, cumbersome and lifelong", the cost of management per thalassaemia patient in Sri Lanka is reported to be SLRs.300, 000 – 400,000 (US$3000 – 4000) per year. Thalassaemia screening programmes have been applied widely in Mediterranean and European countries during the past few dacades. Their success has been based on the excellence of public education programmes about the disease, followed by the development of cost effective and successful mass screening regimens to identify thalassaemia carriers. In Sri Lanka, Beta thalassaemia and Hb E/Beta thalassaemia are the commonest forms of inherited disorders. According to available evidences, there are about 2 – 3 % of Beta Thalassaemia carriers in Sri Lankan population but, within the area belongs and surrounds to North Western Province (NWP), the number of reported thalassaemia cases are high in number compared to other areas of the country. At present, there are about 2000 thalassaemia patients reported and about 100 thalassaemia patients are born in Sri Lanka every year. Out of those 100 thalassaemic births, annually there are about 40-50 new thalassaemia births reported in NWP. Under National Thalassaemia Prevention Programme of the Ministry of Health, Sri Lanka, the whole island was divided into 4 catchment areas. Under those catchment areas 4 special thalassaemia screening units were established in 2010. The screening unit which was established in National Thalassaemia Center at Teaching hospital, Kurunegala has become the most successful among all. It mainly covers NWP and surrounding districts, where thalassaemia is highly prevalent. In addition to cascade screening, National Thalassaemia Committee of Sri Lanka recommended a cut off age for the target population in mass screening as above 14 years of age including school children, unmarried people and couples who are getting ready for their marriage. Furthermore, National committee has deployed a special thalassaemia screening protocol, special colour coded card system and the concept on scientific way of matching horoscopes called 'Rudira Porondama' or horoscope of blood'. (concept of getting a blood test done for thalassaemia carrier status before marriage). All mentioned strategies are considered to be more suitable, realistic and importantly cost effective for a low income generating country like Sri Lanka.
Method. The mass screening is done basically in daily clinic basis and using frequent field visits conducted by Thalassaemia Screening Unit (TSU) of National Thalassaemia Center (NTC), Sri Lanka. It is done according to the screening guidelines initially developed by Thalassaemia eradication programme of NWP and further validated by National Thalassaemia Technical Committee of National Thalassaemia Prevention Programme, Ministry of Health of Sri Lanka.

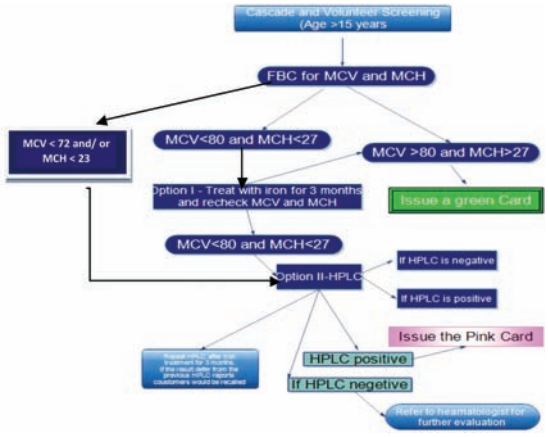

Figure1. Thalassaemia screening protocol. (Source. National thalassaemia prevention programme)

According to the guidelines, after taking written consent from the participants, thalassaemia screening is done using Full Blood Count (FBC) as the preliminary screening test and High Performance Liquid Chromatography (HPLC) as the confirmatory test.

Main theme. Since 2009 mass screening has been carried out continuously aiming basically the target population. It has been intensified gradually over the period. Parallel to the mass screening programme, well organized thalassaemia awareness programme has also been conducted. During this period 71,968 individuals were screened for thalassaemia carrier status. According to the screening protocol, non thalassaemia carriers were issued a green card (called '*Haritha Patha')* and identified thalassaemia carriers were issued a pink card(called '*Roosa Patha')*. Premarital couples were subjected for screening under the theme of checking their horoscope of blood and if both partners were pink card holders they were subjected to special premarital counseling conducted by well trained counselors. According to the data of the mass screening programme, percentage of thalassaemia carriers were 08% out of screened population. Nearly 25% of screened population were subjected to iron treatment for 3 to 6 months. One of the major difficulty encountered during the programme were not having a low cost method for testing serum iron(serum ferritin is expensive test to be used in a screening program) to ensure the people with iron deficiency who had their preliminary MCV <80 and MCH <27. Therefore, that category of people were subjected to 3 months iron treatment regime but, the compliance and the percentage of people returning back after iron treatments to follow rest of the protocol were not up to the expected level. Scarcity of resources (screening machines, reagents and man power), transportation difficulties and other logistic problems were encountered specially during the field screening visits. Under the mass screening programme still National Thalassaemia Center of Sri Lanka has succeeded about 20% reduction in number of new cases reported compared to last few years. In addition to the area coverage problems, not having a compulsory provision to produce thalassaemia screening cards at the time of marriage, if a high risk couple (two pink card holders) wishes to go ahead with their marriage even after premarital counseling, lack of prenatal diagnostic facilities and provisions for legalized abortions are vital issues identified, to be further discussed and assessed.

Conclusion. Properly established efficient and cost effective mass screening programme is considered as an essential component in Thalassaemia eradication process from Sri Lanka. It should be further strengthened by a well designed public awareness programme with multisectoral participation and legal provisions for premarital screening. Though, prenatal diagnosis and legalized abortions can be debatable issues under the cultural background of Sri Lanka, the experiences and the success stories in certain countries are highly influential towards an open forum discussion. Introducing a low cost way of assessing the iron deficiency is also very helpful in the process. Therefore, National Thalassaemia Programme of Sri Lanka should be empowered and further strengthened with the vision of eradication thalassaemia from Sri Lanka in near future.

GLOBIN LENTIVIRAL TRANSFER IN SOMATIC VERSUS INDUCED PLURIPOTENT STEM CELLS OF A β-THALASSEMIA GENE THERAPY PATIENT

Alisa Tubsuwan,[1,2,4] Soumeya Abed,[1,2,3] Cynthia Bartholomä,[5] Melanie Kardel,[6] Annette Deichmann,[5] Alice Cheung,[6] Olivier Negre,[1,2,7] Marine Granger-Locatelli,[1,2] Beatrix Gillet-Legrand,[1,2,7] Zahra Kadri,[1,2] Suthat Fucharoen,[4] Emmanuel Payen,[1,2] Kristof Von Kalle,[5] Manfred Schmidt,[5] Connie Eaves,[6] Stany Chrétien,[1,2] Philippe Leboulch,[1,2,8] Leïla Maouche-Chrétien[1,2]

[1]CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI), Fontenay aux Roses, France; [2]Inserm U962 and University Paris 11, CEA-iMETI, Fontenay aux Roses, France; [3]University Paris 7, France; [4]Thalassemia Research Centre, Institute of Molecular Biosciences, Mahidol University, Nakornpathom, Thailand; [5]Department of Translational Oncology, National Center for Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Heidelberg, Germany; [6]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada; [7]Bluebird bio-France, CEA-iMETI, Fontenay aux Roses, France; [8]Harvard Medical School and Genetics Division, Department of Medicine, Brigham & Women's Hospital

Introduction. Patients with β-thalassemia major require lifelong transfusions and iron chelation, regardless of the type of causative mutations (*e.g.*, β^0, β^E/β^0). The only available curative therapy is allogeneic hematopoietic transplantation, although most patients do not have an HLA-matched, geno-identical donor, and those who do still risk engraftment failure and graft-versus-host disease. Hence, gene therapy by *ex vivo* transfer of a functional β-globin gene into the patient's own somatic hematopoietic stem cells (HSCs) is an attractive novel therapeutic modality. Lentiviral vectors have proven

especially suited for this application because they are capable of both transducing G1-arrested HSCs and carrying a large DNA payload, which is required for inclusion of the complex regulatory elements to express the β-globin gene. Hence, the first Phase I/II human clinical trial was initiated by our group, and the first treated β^E/β^0-thalassemia patient previously suffering from a severe clinical form has become transfusion independent for the past 3.5 years (*Nature* 2010). However, potential oncogenic genotoxicity in some patients remains a concern due to the intrinsic quasi-random nature of lentiviral integration. This is why globin lentiviral transfer to human induced pluripotent stem cells (iPSCs) is an attractive alternative, because one can isolate sub-clones where the vector has integrated in relatively "safe" areas of the human genome. However, a multitude of unknowns and hurdles remain before iPSCs becomes a viable approach, and comparing HSCs derived from human iPSCs with their natural isogenic somatic counterparts had not been performed in the context of therapeutic gene delivery.
Materials and Methods. Mesenchymal stem cells from a β^E/β^0-thalassemia patient who had been treated with gene therapy were reprogrammed into iPSCs by transduction of retroviruses carrying 4 transcription factors: Oct-4, Sox-2, Klf-4, and c-Myc. iPSC subclones were then transduced with the same batch of β-globin lentivector used in the clinical trial. Lentivector integration sites were then identified by DNA pyrosequencing and compared with those in the isogenic patient. Ability of the globin lentivector-transduced iPSCs to differentiate into hematopoietic cells *in vitro* and *in vivo* was investigated by coculture with stromal cells and transplantation into immunocompromised mice. Globin gene expression was determined by qRT-PCR and HPLC analyses.
Results. After transducing the gene therapy thalassemia patient's iPSCs with the same globin lentivector, high vector-encoded β-globin expression, chromosomal integration in regions of low and high genotoxicity, multi-lineage engraftment in immunocompromised mice, and embryonic-fetal together with partial fetal-adult globin class switching were observed. Surprisingly, common integration sites were identified across iPSC lines and cells retrieved from isogenic and non-isogenic gene therapy patients with β-thalassemia and adrenoleukodystrophy, respectively. Common integration sites observed in the absence of overt tumorigenesis thus results from non-random lentiviral integration rather than oncogenic *in vivo* selection.
Conclusion. These findings bring the use of human iPSCs closer to practicality and further clarify both mechanics and interpretation of genome-wide lentivector integration.
Keywords. human induced pluripotent stem cells (iPSC), gene therapy, β-thalasasemia, lentiviral vector integration.
Acknowledgment. AT was supported under Royal Golden Jubilee-Ph.D program from Thailand Research Fund and the CEA/iMET/STI, France.

CURCUMA LONGA LINN. ENHANCED γ-GLOBIN EXPRESSION AND HEMOGLOBIN F PRODUCTION

Nattawara Chaneiam,[1,2] Chatchawan Changtam,[3] Apichart Suksamrarn,[3] Pranee Winichagoon,[2] Soavaros Svasti,[2] and Suthat Fucharoen[2]

[1]Graduate Program in Nutrition, Institute of Nutrition, Mahidol University, Thailand; [2]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Thailand; [3]Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Thailand

Introduction. Pathology of β-thalassemia occurs from the excess unbound α-globin chain. An alternative treatment is to stimulate γ-globin chain production, which is able to bind to the unbound α-globin to form Hb F. Our previous study has shown that extracts from *Curcuma longa* Linn., bisdemethoxycurcumin enhanced γ-globin expression and HbF production in erythoid cells line (3.9 ± 0.1 and 1.38 ± 0.1 folds, respectively). In this study, HbF enhancing activity of bisdemethoxycurcumin and its reduced form, tetrahydro- and hexahydrobisdemethoxycurcumin, were evaluated in human erythoid progenitor cells.
Materials and Methods. Peripheral blood $CD34^+$ cells from healthy volunteers were isolated by immunomagnetic positive selection, and cultured in two-phase media. Isolated $CD34^+$ cells were resuspended at a density of 1×10^5 cells/ml in the first phase media containing IMDM supplemented with 30%FBS, 0.01% BSA, 50 ng/ml SCF, 20 ng/ml IL-3, and 0.1 U/ml EPO. On day 7, cells were adapted into the second phase media (IMDM supplemented with 30% FBS, 0.01% BSA, 5 U/ml EPO, and 0.1 ng/ml IL-3) in the absence or presence of compounds. The levels of γ-globin mRNA and HbF induction were determined on day 14 by RT-qPCR and flow cytometry, respectively.
Results. The results showed that 20 μM tetrahydro- and hexahydrodemethoxycurcumin enhanced γ-globin gene expression as indicated by the reduction of α/γ globin ratio and the effect was more pronounced than parent compound, bisdemethoxycurcumin. Furthermore, 20 μM tetrahydro- and hexahydrobisdemethoxycurcumin increased 2.46 and 2.19 folds of HbF production, respectively when compared with untreated. All compounds (final concentration <30 μM) had low toxicity (>70% viable cells).
Conclusion. Biochemical and its metabolites from *C. longa* Linn. have potential to be developed as an alternative new therapeutic agent for HbF enhancer in β-thalassemia patient.
Acknowledgements. This study was supported in part by the Office of the Higher Education Commission and Mahidol University under the National Research University Initiative, Thailand Research Fund and National Science and Technology Development Agency, Thailand.

TELOMERE SHORTENING IN β-THALASSEMIA/HB E PATIENTS

Porntip Chaichompoo, Saovaros Svasti, Pranee Winichagoon and Suthat Fucharoen

Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand

Introduction. The clinical manifestation of β-thalassemia/Hb E patients is heterogeneous ranging from mild cases, the intermedia and severe cases with transfusion-dependent conditions. The excess unbound α-globin

chains, which precipitate in the red cell precursors and red blood cells, lead to highly reactive oxygen species (ROS) generation and cell destruction in the bone marrow and circulation. This process causes severe anemia, which in turn leads to increased erythropoiesis. During DNA replication of linear DNA, DNA polymerase cannot replicate DNA at the end of a linear chromosome (telomere). Consequently, the telomeres are shortening in every cycles of cell division. In this study, the correlation between telomere shortening and oxidative stress in β-thalassemia/Hb E patients were observed, supporting. The "free radical theory of aging" that oxidative stress may promote the telomere shortening.

Methodology. 30 β-thalassemia/HbE patients, divided into 3 groups accordingly to severity, and 15 healthy subjects were recruited. The relative telomere length (RTL) was determined in peripheral blood mononuclear cells using Flow-FISH (FITC conjugated with telomere/PNA probe, Dako). Oxidative stress assays, fluorescein dyes, which react to ROS, GSH, and mitochondrial membrane potential and detected by flow cytometry.

Results and Discussion. We observed the aging-dependent in all individuals, both controls and β-thalassemia/Hb E patients. Interestingly, the RTL analysis of the matching age (20-30 year-old) subjects, between patients and the individual healthy controls, showed severity-dependent in the patients. The telomere length of severe patients was significantly shortening when compared to healthy subjects (p=0.001). Additionally, there was a correlation between either the percentages or the absolute numbers of reticulocyte counts and RTL (r_s=0.535 and r_s=0.511 for p <0.001, respectively). The ROS levels in lymphocytes obtained from severe patients may correlate with telomere erosion (r_s=0.406, p=0.191). Additionally, the GSH level of leukocytes from patients was significantly lower than these of healthy subjects (p <0.05). Meanwhile the mitochondrial membrane potential analysis between groups in this cohort was not different. This investigation supports the hypothesis that the high level of oxidative stress and the low level of anti-oxidant in thalassemic cells could risk to telomere erosion

Conclusion. This investigation is the first initiation to understand how telomere erosion in β-thalassemia/Hb E disease.

Keywords. telomere, thalassemia, oxidative stress, flow-FISH, flow cytometry

Acknowledgments. This work was supported by Mahidol University; PC was supported under Post-doctoral fellowship program, Mahidol University Research Grant.

AUTOPHAGIC CELL DEATH IN MURINE β-THALASSEMIC ERYTHROID PRECURSOR CELLS

Ramaneeya Nithipongvanitch,[1] Porntip Chaichompoo,[1] Kanitta Srinoun,[1] Duncan R. Smith,[2] Suthat Fucharoen,[1] Saovaros Svasti[1] and Pranee Winichagoon[1]

[1]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Thailand and [2]Molecular Pathology Laboratory, Institute of Molecular Biosciences, Mahidol University, Thailand

Introduction. The pathophysiology of β-thalassemia occurs from excess unbound α-globin chains, which precipitate and cause oxidative damage in erythroid progenitor cells, leading to intramedularly destruction (ineffective erythropoiesis) and anemia. Ferrokinetics studies in b-thalassemic patients demonstrated that as many as 80% of the erythroid progenitors die in the bone marrow. It is believed that apoptosis is an important process of ineffective erythropoiesis. However, the exact mechanism of the death process of erythroid precursor cells is unknown. This study aims to examine the mechanism of erythroid progenitor cell death using thalassemic mice bone marrow as a model.

Methodology. Apoptotic and autophagic responses of bone marrow erythroid precursors cells were examined in b-thalassemic and wild type (WT) mice based on flow cytometry, Western blotting assay, transmission electron microscope (TEM), immunogold EM, and co-localization analysis.

Results and Discussion. A significant increase of the amounts of phosphatidylserine-bearing basophilic erythroblasts in β-thalassemic mice was observed when compared to WT mice. However, there was no evidence of other apoptosis characteristics such as caspase activation, loss of mitochondrial transmembrane potential, chromatin condensation, and DNA ladder in this animal model. Interestingly, the level of Bcl-X_L was significantly increased in thalassemic erythroid cells when compared to WT mice. Additionally, immature and mature thalassemic erythroblasts containing autophagic vacuoles were significantly increased as measured by TEM when compared to WT. The co-localization between the LC3 protein (an autophagosomal marker), assessed by immunogold analysis under the high resolution EM) and LAMP-1 protein (a marker of endosomes and lysosomes) was confirmed using the indirect immunofluorescent assay and confocal analysis.

Conclusion. These results suggest that an enhanced autophagy at the early stage of erythroid differentiation may be one of the mechanisms involving in ineffective erythropoiesis in b-thalassemic mice.

Keywords. autophagy, apoptosis, ineffective erythropoiesis, thalassemic mice

Acknowledgments. This work was supported by Mahidol University; RN, PC, and KS were supported under Post-doctoral fellowship program, Mahidol University Research Grant.

IMPAIRED BONE FORMATION AND ENHANCED BONE RESORPTION IN HETEROZYGOUS $^{\beta IVSII-654}$ KNOCKIN THALASSEMIC MICE

Kanogwun Thongchote,[1,2] Saovaros Svasti,[3] Mayurachat Sa-ardrit,[4] Nateetip Krishnamra,[1,2] Suthat Fucharoen,[3] Narattaphol Charoenphandhu[1,2]

[1]Department of Physiology; [2]Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Bangkok, Thailand; [3]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Bangkok, Thailand; [4]National Laboratory Animal Center, Mahidol University, Bangkok, Thailand

Introduction. Mutation of β-globin gene from cysteine to tyrosine at nucleotide 654 of the second intron, so called $\beta^{IVSII-654}$, leads to heterozygous aberrant β-globin mRNA splicing mimicking thalassemic intermedia phenotype commonly found in South-East Asia, including Thailand. Although trabecular and cortical bone density was previously reported in β-thalassemia, the cellular changes in trabecular microstructure in $\beta^{IVSII-654}$ thalassemic mice have not been demonstrated. Therefore, the objectives of this study were to demonstrate a coupling of bone formation and bone resorption (bone remodeling) and microstructural changes in tibial metaphysis of $\beta^{IVSII-654}$ thalassemic mice.
Materials and Methods. 12-week-old wild-type C57BL/6 male mice and $\beta^{IVSII-654}$ thalassemic littermates (n=4/group) were obtained from the National Laboratory Animal Center, Thailand. Trabecular microstructure and bone remodeling in proximal tibial metaphysis were evaluated by bone histomorphometry technique.
Results. In $\beta^{IVSII-654}$ thalassemic mice, trabecular bone volume (BV/TV) was significantly decreased while bone marrow cavity was expanded, as indicated by marrow volume (Ma.V/TV). Bone formation in thalassemic mice was reduced as represented by osteoblast surface (Ob.S/BS), osteoid surface (OS/BS), osteoid volume (OV/TV), mineral apposition rate (MAR), mineralizing surface (MS/BS) and mineralized volume (Md.V/TV). Moreover, bone resorption parameters *i.e.*, osteoclast surface (Oc.S/BS) and eroded surface (ES/BS) were significantly increased.
Conclusion. Our thalassemic mouse model demonstrated a bone defect at cellular and microstructural levels leading to reduced bone formation whereas enhanced bone resorption. Thus, it could explain a mechanism underlying osteopenia commonly found in thalassemic patient.
Key words. Bone histomorphometry, Thalassemia, Goldner's trichrome, Osteoblasts, Osteoclasts, Osteoporosis.
Acknowledgement. This work was supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant PHD/0352/2550).

THALASSEMIA IN CAMBODIA

Jatuporn Tanakulmas,[1,2] Thongperm Munkongdee,[1]
Saovaros Svasti,[1] Punnee Butthep,[2]
Pranee Winichagoon,[1] Courtney Anderson,[3]
Robyn Devenish[4] and Suthat Fucharoen[1]

[1]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Thailand; [2]Department of Pathology Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand; [3]World vision Cambodia (WVC), Cambodia; [4]Pediatric Hospital, Phnom Penh, Cambodia

Introduction. Thalassemia is the most common inherited hematological disease that causes major public health burden for many countries in South-East Asian countries including Cambodia. The information about molecular mechanism and prevalence of thalassemia in Cambodia is limited to only some areas leading to a lack of proper prevention and control program. This research project extends the study on prevalence and molecular mechanism of thalassemia in a wider Cambodia population, aiming to achieve more data for the national thalassemia program.
Materials and Methods. One thousand six hundred and thirty-four EDTA blood specimens were collected from 77 villages in 4 provinces. Complete blood counts and hemoglobin typing were performed. α-thalassemia genotyping was examined by gap-PCR and dot blot hybridization. β-thalassemia mutation was detected by reverse dot-blot hybridization using allele specific probes for mutations in Thai.
Results and Discussion. The overall prevalence of thalassemia was 62.79% of the population in this cohort. The genes frequencies of thalassemia were 0.0021 β^{T}-thalassemia, 0.2355 β^{E}, 0.0098 α-thalassemia 1($--^{SEA}$), 0.1603 α-thalassemia 2 ($-\alpha^{3.7}$), 0.0055 α-thalassemia 2 ($-\alpha^{4.2}$), 0.0337 Hb Constant Spring and 0.0125 Hb Paksé.
Conclusion. The results provide a better understanding on epidemiology of these abnormal genes that may be used to plan for thalassemia prevention and control program in Cambodia.
Keywords. thalassemia, DNA analysis, epidemiology, prevention and control, Cambodia
Acknowledgements. This work was supported by Mahidol University Research Grant, Thailand and World vision Cambodia (WVC), Cambodia.

THALASSEMIA AND ABNORMAL HB AMONG MUONG ETHNIC PEOPLES IN THE NORTH OF VIET NAM

Duong Ba Truc, Bui Van Vien, Nguyen Hoang Nam, Nguyen Thi Ha

The national hospital of pediatric Ha Noi Viet Nam

Prevalence of Thalassemia in Viet Nam differs from one ethnic groups to others. While among Kinh, the largest group,the prevalence of β Thalassemia is about 2-4 %, among some minority groups it is up to 20-30%. Muong is the 3rd largest group of minority ethnics in the North of Viet Nam (with a population about 1,5 milions). The purpose of the study was held with purpose to find out the prevalence of Thalassemia among Muong people in Hoa Binh province, and to make a plan for Thalassemia prevention in the province.
Object and Method. 1890 clinically healthy Muong people aged from 15-35 years Kim Boi district, Hoa Binh province were involved in this study. The subjects was collected without any selection. From each person, 2ml blood was taken to do the following test: OF test with 0,35% NaCl solution; CBC test automatic hematology analyzer (Beckman Coulter); Hb – HPLC test variant (Bro Rad); 24 children with clinical symptoms of Thalassemia were also examined to find out the diagnosis.
Results. Among children – patients 8/24 have HbE/β Thal; 9/24 have HbH desease and 7/24 have β Thal severe or intermediate. Among 1890 clinicaly healthy people we found: 190 were carriers for HbE (10,1%), 169 were carriers for β Thalassemia (9,4%) and 2 were carriers for Hb D. A further 197 people were found to have microcytosis and hypochromia without changes in Hb.

Conclusion. The prevalence of β Thalassemia and HbE among Muong ethnic people in Hoa Binh is very high, up to 19,5% (HbE 10,1% and β Thal 9,4%). This is reflect in the high number of pediatric patient with HbE/ β Thal, and β Thal severe or intermediate. The number of children with HbH disease was high (9 patients). Many asymptomatic people have microcytosis and hypochromia, but in the study we can not discriminate them as α Thal carriers or iron deficiency. Two patient were HbD carriers.

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