INVITED REVIEW ARTILCLE

Genotype-Phenotype Diversity of Beta-Thalassemia in Malaysia: Treatment Options and Emerging Therapies

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The haemoglobinopathies and thalassemias represent the most common inherited monogenic disorders in the world[1]. Beta-thalassaemia major is an ongoing public health problem in Malaysia[2]. Prior to 2004, the country had no national policy for screening and registry for thalassemia. In the absence of a national audit, the true figure of the extent of thalassemia in the Malaysian population was largely presumptive from micro-mapping studies from various research workers in the country. The estimated carrier rate for beta-thalassemia in Malaysia is 3.5-4%. There were 4768 transfusion dependent thalassemia major patients as of May 2010 (Data from National Thalassemia Registry).

KEYWORDS:
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PATHOPHYSIOLOGY OF ANEMIA IN BETA-THALASSEMIA

Thalassemia is a disorder of haemoglobin synthesis which is characterized by the absence or reduced synthesis of globin chains, α,β,δ,γ,ζ and ε of human haemoglobin (Hb). The two main types of thalassemia are α and β-thalassemia. Phenotypically there two forms of β-thalassemia: β^0 – no β globin chain synthesis, and β^+ - with some β globin chain synthesis. Clinically, thalassemia presents as beta-thalassemia trait (minor) (β^+ or β^0), intermedia (β^+/β^+; β^+/β^0) or major (β^0/β^0). Beta-thalassemia trait (minor) is usually asymptomatic and is associated with the inheritance of a single gene defect. Beta-thalassemia major results in severe transfusion dependent anemia and is caused by the inheritance of two beta-globin gene mutations either in a compound heterozygous or homozygous state. Beta-thalassemia intermedia is of moderate severity and the majority of affected individuals do not require regular blood transfusions[3].

In beta-thalassemia the synthesis of the alpha globin chains continue despite the absence or reduced synthesis of the beta-globin chains of human haemoglobin. This imbalance of the globin chains is the cause of the anemia in thalassemia. The excess alpha globin chains precipitate in the developing red blood cells in bone marrow and in the peripheral blood resulting in their damage and reduced red cell survival. Inclusion body formation and oxidative damages to developing red blood cells in the bone marrow leads to ineffective erythropoiesis which is the hallmark of thalassemia. Red blood cells formed that are able to make their way into the peripheral blood also get destroyed in a similar way resulting in peripheral hemolysis. The beta-globin chain deficit is 50% for beta-thalassemia trait (minor), 100% for beta-thalassemia major and variable between 50-80% for beta-thalassemia intermedia. Although in beta-thalassemia trait, the beta-globin chain production deficit is 50%, the Hb is normal or mildly reduced. The excess alpha globin chains are removed by proteolysis and compensation is seen by increased erythropoesis.

Anaemia is the most common condition seen in clinical practice in all age groups. Red blood cell production requires an adequate supply of iron, folate, vitamin B12 and pyridoxine. The cause of the anaemia encompasses nutritional deficiencies including the underlying disease process. Anaemia of chronic disease is commonly seen in hospital patients. In surgical patients and those with disease processes where blood loss is featured, iron deficiency anemia is seen. In oncology patients, including those with haematological diseases, marrow infiltration alters hemopoiesis and chemotherapy results in suppression. Nutrition in addition may be compromised in oncology patients[4]. In populations where thalassemia is prevalent, iron deficient red blood cell indices may be confused with thalassemia indices. Classical beta-thalassemia trait has hypochromic microcytic red cell indices and a raised HbA_2 with values 4% and above when measured by high performance liquid chromatography (HPLC)[5-8]. In beta-thalassemia intermedia and major the red cell indices are also hypochromic and microcytic. However the peripheral blood film has profound red cell morphological changes. HbF ($\alpha_2\gamma_2$) is raised and no HbA ($\alpha_2\beta_2$) is synthesized in beta-thalassemia major. In beta-thalassemia intermedia and major, the serum ferritin is raised or may be normal in the presence of iron chelation therapy[9]. In classical beta-thalassemia carriers, the serum ferritin levels are normal[10]. However, carriers can show functional iron deficiency[11]. In pregnancy iron supplementation may be required in beta-thalassemia carriers.

GENETIC MODIFIERS

The major genetic modifiers of β-thalassemia are the genotypes of the β- and α- globin and expression of γ-globin.

The spectrum of beta-thalassemia alleles has been determined in a wide variety of population groups. At present, more than 200 different beta-thalassemia mutations have been described. List of mutations causing β-thalassemia is available at the globin gene server (http://globin.cse.psu.edu). The large majority of mutations causing β-thalassemia are primarily point mutations and others include deletions or addition of nucleotides that involve the beta-globin gene complex. Expression of the beta-

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globin genes may be affected by mutations in the regulatory regions upstream of the beta-globin gene complex. Population studies demonstrated that approximately 25 mutations represent the vast majority of the β-thalassemia alleles in all populations at risk. A small number of ethnic/population group-specific alleles account for about 70-90% of the beta-thalassemia genes while a larger number of rarer alleles have been observed in each ethnic group that account for the remaining genes[12-13].

The total population in Malaysia is estimated as 28 million. Malaysia is multiracial with different religions and cultural beliefs. The three main races are the Malays (65%), Chinese (26%) and Indians (8%). In addition there are Ceylonese, Indonesians, Pakistani, Myanmar, Thais and Europeans. Indigenous people are present in Peninsular Malaysia (Orang Asli) and in East Malaysia (Sarawak and Sabah). Each ethnic group has its characteristic set of beta-thalassemia mutations.

The spectrum of beta-thalassemia mutations in Malaysia have been systematically delineated since 1984. Three common β-globin mutations seen in the Malays, HbE [CD 26 (CAG→AAG)], IVS 1-5 (G→C) and IVS1-1 (G→T) were responsible for about 73.1% of β-thalassemia. HbE and IVS1-5 (G→C) both have β^+-thalassemia phenotype. There are five common β-globin mutations in the Chinese-Malaysians: CD 41/42 (-TCTT), IVS2-654 (C→T), -28 (A→G), CD 17 (A→T) and CD71/72 (+A) and these account for about 90% of β-thalassemia. All have β^0-thalassemia phenotype except IVS2-654(C→T) and -28 (A→G) which have β^+-thalassemia phenotype. In the Kadazan-Dusun of Sabah the most common mutation found in over 90% of transfusion dependent thalassemia patients is the 45 kb Filipino deletion. This latter mutation has β^0-thalassemia phenotype[14-30].

Alpha globin chains in beta-thalassemia can combine with gamma and delta globin chains to form HbF ($\alpha_2\gamma_2$) and HbA_2 ($\alpha_2\delta_2$) respectively. However this expression is affected by factors that modulate alpha globin chain excess and its stability. Genetic factors include the specific beta-thalassemia mutation, alpha globin gene dosage, gamma chain expression and membrane disorders. The primary beta globin gene mutation expression as β^+ is β^0 is a critical factor. Mild mutations have less reduction in beta-globin chain production resulting in some HbA ($\alpha_2\beta_2$) formation whereas in beta-thalassemia major (β^0/β^0) there is no beta globin chain formed at all and the affects of deleterious access of alpha globin chains are clearly seen. Compound β^E/β^0- thalassemia is the most common form of severe beta-thalassemia in southeast Asian countries. The β^E-globin allele bears a point mutation that causes alternative splicing. The abnormally spliced form is non-coding producing no β^E. The correctly spliced messenger RNA expresses a mutated β^E-globin with instability. Interaction with a non-functional β^0 allele results in profound decrease in β-globin chain synthesis. About 50% of β^E/β^0- thalassemia patients are transfusion dependent.

A normal person has four alpha globin genes producing alpha globin chains. Thus, alpha thalassemia ameliorates beta-thalassemia by reducing the excess alpha globin chains whereas presence of more than 4 functional alpha globin genes aggravates the condition. This latter status results in the production of more alpha globin chains contributing to excess of alpha globin chains in beta-thalassemia. The concurrent inheritance of α-thalassemia 1 ($--^{SEA}$)(α^0-thalassemia) with beta-thalassemia was seen in 3.5%[31-33]. Concurrent inheritance of deletional α-thalassaemia in Malays with HbE trait was seen in 11.1% where the most prevalent interaction was with α^+-thalassemia ($\alpha^{-3.7}$). Only 2.2% had the α^0-thalassemia molecular defect[34-35].

The stability of unpaired alpha globin chains is modulated by alpha-haemoglobinstabilizing-protein (AHSP). AHSP has been described to play an important role in erythropoiesis. It is involved in folding of the alpha-globin chains for beta-globin association, heme binding, transfer for beta-globin association and stabilization of alpha-globin chains. Mice lacking AHSP have abnormal red cell production and lifespan. AHSP is a specific molecular chaperone that binds alpha-globin chain of haemoglobin and prevents alpha globin chain precipitation[36-37]. AHSP inhibits reactive oxygen species (ROS) production from alpha-globin chain excess[38].

Reduced AHSP can mean reduced protection from stressors such as fever, oxidizing conditions and presence of toxins. In mice, the phenotype of beta-thalassemia intermedia is exacerbated by concomitant loss of AHSP[39]. However studies in humans with thalassemia produced variable data on AHSP as a modifier of beta-thalassemia phenotype suggesting that there may be population variability of AHSP expression. GATA-1 and Oct-1 are regulatory elements required for the expression of the human AHSP gene. The AHSP promoter region is an excellent candidate region for mutations associated with decreased or increase AHSP gene expression[40]. AHSP expression in HbE-beta-thalassemia patients varied up to 1.52 –log differences in a cohort of patients studied in West Malaysia[41]. Studies in patients with mild, moderate and severe HbE beta-thalassemia in Thailand suggested that AHSP is not a disease modifier in this form of thalassemia[42].

Human Hb production is characterized by two major `switches': production of embryonic Hb (Hb Gower 1 ($\alpha_2\varepsilon_2$); Hb Gower 2 ($\zeta_2\varepsilon_2$) and Hb Portland ($\zeta_2\gamma_2$) and swithches after 2 months of gestation to production of two types of fetal Hb ($\alpha_2{}^G\gamma_2$ and $\alpha_2{}^A\gamma_2$) and just before birth to adult Hb A ($\alpha_2\beta_2$). At 6 months after birth, HbF comprises less than 5% of total Hb and reaches adult level at 2 years of age[43]. Clinically severe forms of beta-thalassemia become apparent on completion of the switch from fetal to adult Hb. The majority of beta-thalassemia major patients will present in the first year of life and the rest in the second. There is considerable variation in the amount of HbF levels in normal adults. Factors known to influence HbF levels are age, sex, inheritance of β thalassemia and genetic factors such as DNA sequence variations within the β globin gene cluster as well as genes unlinked to the β globin gene cluster[44]. Increased HbF levels or F-cell (HbF containing erythrocyte) numbers can ameliorate the disease severity of β-thalassemia major.

Increased HbF levels may occur as a direct or indirect effect of genetic disorders. Inherited disorders primarily associated with increased HbF levels due directly to increased production fall into two groups – hereditary persistence of fetal hemoglobin (HPFH) and δβ-thalassemia. Genetic disorders indirectly associated with increased HbF levels are the beta-thalassemias. β-thalassemia trait usually have normal or slightly increased HbF levels. The range of HbF levels in homozygous β-thalassemia range from 10% in mild alleles (β^+) and to about 100% in those with β^0 alleles. A sequence variation (C→T) at position -158

upstream of the $^{G}\gamma$ globin gene has been shown to increase HbF levels in both normal individuals and in patients with beta-thalassemia [45-46]. Studies in Thailand indicate that the HbF level increase is more significant when this gene polymorphism is inherited in a homozygous state. In Malays, heterozygosity of the Xmn 1 site (+/-) was most common and seen in 63.3% of patients with beta-thalassemia. Homozygosity for the Xmn 1 site (+/+) was absent in the Chinese-Malaysians but identified in 8.2% of the Malay patients. Homozygosity for the Xmn 1 (-/-) was seen in 89.7% of Chinese-Malaysian patients with beta-thalassemia major[47].

Recent genome-wide association studies reported that single nucleotide polymorphisms (SNPs) in the BCLL11A gene on chromosome 2p16.1 were correlated with F-cells among healthy Europeans, and HbF among Sardinians, Chinese and Thais with beta-thalassemia. BCL11A is a major Hb quantitative trait locus in populations with β-thalassemia. Data suggest that functional motifs responsible for modulating F-cells and HbF levels reside with a 3 kb region of the BCL11A gene[48].

Hereditary ovalostomatocytosis is a common inherited membrane disorder present in 5.1% of Malays[49]. Ektacytometric studies of peripheral blood show membranes of red blood cells in hereditary ovalostomatocytosis are markedly rigid[50-51]. The clinical effects of the genes of beta-thalassemia and hereditary ovalocytosis are summated in an aggravation of haemolysis[52].

TREATMENT OPTIONS AND EMERGING THERAPIES

Beta-thalassemia carriers are asymptomatic and do not require blood transfusions. However functional iron deficiency may occur in this group and treatment by hematinics. In the majority of patients with beta-thalassemia intermedia blood transfusion may be required in fulminant infections, during pregnancy and when drugs with oxidant properties are administered. In severe beta-thalassemia (thalassemia major), unbalanced globin chain synthesis produces extensive destruction of immature red cells in the bone marrow (ineffective erythropoiesis) and mature red blood cells in the peripheral blood (peripheral hemolysis) resulting in anemia. Anemia provokes compensatory hyperplasia of the erythroid marrow. Ferrokinetic studies indicate erythroid proliferation may exceed about 10-20 times basal level. Additional deleterious effects caused by the erythroid bone marrow expansion include increased iron absorption, extramedullary hemopoietic masses and hypercatabolic state. Studies indicate that a transfusion program of regular monthly blood transfusions of packed red blood cells keeping a baseline Hb of 9-10 g/dl suppresses erythropoiesis[53-54].

In Malaysia, the conventional mode of therapy in beta-thalassemia major patients is regular monthly transfusions for life. Patients require iron chelation to remove the excess iron that accumulates in the body from both the blood transfusions and from increased iron absorption from the gastrointestinal tract[55].

Allogeneic hemopoietic stem cell (HSC) transplantation is currently the only treatment with curative potential for severe beta-thalassemia. Stem cells are sourced from umbilical cord blood, peripheral blood and bone marrow of human leukocyte antigen (HLA) matched donors[56-57]. Facilities are available in four centres in Malaysia for HSC transplantation[58-59]. A total of 144 transplants for thalassemia have been recorded from 1997 to 2008. [The National Transplant Registry; University Malaya Medical Centre contributed 98 cases: Data from Professor Chan LL (personal communication)]. HSC source consisted of compatible bone marrow and umbilical cord. The overall survival rate is 82%. The cure rate does not differ by more than 5% from survival rate.

The only established curative therapy for beta-thalassemia major is allogeneic stem cell transplantation from a matched related donor. However, at least 70% of these patients lack such a donor. An emerging treatment is gene therapy for gene transfer using lentoviral vectors. Experiments in animal models, mice with beta-globin mutations have demonstrated the efficacy of globin gene transfer using lentoviral vectors. The first human thalassemia patient treated with gene therapy using a lentoviral vector in 2007 was reported as transfusion independent in 2010[60]. Gene transfer of the hepcidin gene (HAMP) to reduce intestinal iron absorption and restoration of the balanced α/β globin gene expression in β654-thalassemia mice using combined RNA and antisense RNA approach have been reported. In β-thalassemia, given the excess of α-globin leads to widespread detrimental effects, it was found that using the RNAi pathway to mediate reduction in α-globin expression has potential as a therapeutic strategy[61-62].

Increased HbF synthesis ameliorates beta-thalasemia. Reactivation of γ-chain synthesis is a tangible approach showing beneficial effects. In β-thalassemia the additional γ chains bind to the α-chains remaining in excess because of absence of their normal partners the β-chains: This result in ameliorating the deleterious effects of intracellular precipitation of excess of α-chains and production of some functional HbF in the developing red cell precursors occurs: Ineffective erythropoiesis is reduced and the red cells survive longer in the circulation[63]. Coinheritance of hereditary persistence fetal hemoglobin (HPFH) reduces clinical severity. Pharmacological manipulation has been approached to increase Hb F production. 5-azacytidine, sodium 4 phenylbutyrate and hydroxyurea are compounds able to do this. However 5-azacytidine is potentially carcinogenic and restricted to end stage β-thalassemia patients where transfusion therapy is not possible due to development of antierythrocyte antibodies or intractable iron overload[64]. A number of clinical trials have used hydroxyurea either alone or with erythropoietin to maintain transfusion independence or reduce transfusions. Studies with beta-thalassemia with hydroxyurea indicate however the increase in HbF levels may not be adequate to maintain transfusion independence [65-66].

SUMMARY

In Malaysia, best possible care is provided to beta-thalassemia major patients by conventional treatment of optimal regular monthly transfusion and iron chelation. Cure through hemopoietic stem cell transplantation being offered to a limited few where a HLA matched compatible sibling donor is available. Improved understanding of the pathophysiology of beta-thalassemia in the last two decades indicates that gene therapy provides the potential for molecular therapies. Molecular analysis of patients with thalassemia to identify gene modifiers will improve genetic counselling and clinical management in Malaysia.

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