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Recent developments in the treatment of transfusion dependent thalassaemia

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Thalassaemia is one of the most common genetic disorders in the world which is particularly common in tropical countries including Sri Lanka [1]. Worldwide, approximately 70,000 children are born with thalassaemia each year. Of all forms of thalassaemia, β-thalassaemia major and haemoglobin E (HbE) β-thalassaemia are the most prevalent and clinically significant sub-types. In Sri Lanka, approximately 1800 patients with thalassaemia receive treatment at present, of which 70% has β-thalassaemia major while 20% has HbE β-thalassaemia [2, 3].

Over 250 mutations of the human β-globin gene could result in β-thalassaemia. β-Thalassaemia major is caused by inheritance of the same β-thalassaemia mutation (homozygous) or two different mutations (compound heterozygous) in both alleles of the β-globin gene [4]. In contrast, HbE β-thalassaemia is due to co-inheritance of a β-thalassaemia mutation in one allele and β^E mutation in the other allele. β^E mutation is a point mutation that results in a structurally abnormal functional haemoglobin, HbE ($\alpha_2\beta^E{}_2$) which is synthesised at reduced rates [5].

Patients with severe forms of β-thalassaemia present during the first year of life with lethargy, poor feeding, failure to thrive, pallor, hepatosplenomegaly and bony changes. These patients require regular transfusions and are transfusion dependent for life. To counteract for resultant iron overload, they are also prescribed iron chelator medications [6]. Allogenic haemato-poietic stem cell transplantation (HSCT) remains the only available cure for β-thalassaemia, however, is not recommended to a majority of patients due to limitations in donors and serious adverse effects. Therefore, a vast majority of patients with β-thalassaemia are managed exclusively with medical treatment, in the developing countries in particular. In this article, I intend to highlight the new advances in the medical management of β-thalassaemia and to summarise promising novel therapeutic options which are being developed.

Transfusion therapy for patients with β-thalassaemia is currently governed by the guidelines published by the Thalassaemia International Federation (TIF). These guidelines recommend maintaining pretransfusion haemoglobin between 9.0-10.5g/dL by transfusing leucodepleted packed red blood cells (RBC) every 2-5 weekly [7]. The aims of transfusion are to correct

anaemia and its compilations, suppress bone marrow activity and extramedullary erythropoiesis and promote normal growth. Although these recommendations are universally accepted and practical for patients with β-thalassaemia major, they do not provide sufficient guidance to the management of HbE β-thalassaemia.

Unlike β-thalassaemia major, the clinical severity of HbE β-thalassaemia is extremely variable. Depending on the amount of HbE synthesised, the severity varies from a minor disease which does not require transfusions through a milder non-transfusion dependent thalassaemia requiring occasional transfusions to severe transfusion dependent thalassaemia that need regular transfusions [8]. Due to this heterogeneity, it is difficult to devise uniform guidelines; thus, the clinicians caring for these patients use different target pre-transfusion haemoglobin levels and diverse transfusion regimens. This non-uniform practice has resulted in serious adversities and led to sub-optimal management of patients with HbE β-thalassaemia.

The magnitude of this hazard is highlighted in our recent multicentred study among 328 patients with transfusion dependent β-thalassaemia [9]. This study revealed that only 4% of patients with transfusion dependent HbE β-thalassaemia had maintained optimal pretransfusion haemoglobin level compared to 47% of patients with β-thalassaemia major. Furthermore, a significantly higher proportion of patients with transfusion dependent HbE β-thalassaemia had hepatosplenomegaly compared to β-thalassaemia major. Nonetheless, patients with transfusion dependent HbE β-thalassaemia were receiving large transfusion volumes similar to that of β-thalassaemia major.

This irony of sub-optimal haemoglobin in spite of receiving high transfusion volumes has made patients with HbE β-thalassaemia vulnerable to both chronic anaemia and iron overload related complications. This is primarily due to clinicians using lower haemoglobin values (usually 7g/dL) as target pre-transfusion haemoglobin level in HbE β-thalassaemia even if patients are transfusion dependent. Therefore, we recommend if patients with HbE β-thalassaemia are transfusion dependent and require transfusions more frequently than 6-weekly, they should be managed identically to β-thalassaemia major by maintaining pretransfusion haemoglobin level between 9-10.5g/dL. However, it should not be confused with a large proportion of patients with mild HbE β-thalassaemia who require 'no' or 'occasional' transfusions. These patients should be managed as non-transfusion dependent thalassaemia and allowed to have low baseline haemoglobin as low as 6-7g/dL without transfusions, provided that they are asymptomatic, have normal growth and does not have rapid enlargement of the spleen.

Iron overload is an inevitable complication of thalassaemia. This is due to two main reasons. Firstly, the patients with thalassaemia have inappropriate suppression of hepcidin synthesis, which in turn promote intestinal iron absorption despite having higher body iron stores. Secondly, with each packet of RBCs, 200mg of iron is infused into the body bypassing the intestinal regulation of iron absorption.

The TIF guideline recommends commencing screening for iron overload using serum ferritin after ten transfusions or when a patient reaches two years. The threshold to start iron chelation is serum ferritin >1000ng/mL. To assess the practicality and usefulness of this recommendation, we recently performed a detailed analysis of trends of iron overload in a group of children with β-thalassaemia [10]. This revealed a rapid rise in iron loading in children within the first five years, and especially during the first two years of life. Importantly serum ferritin was above 1000ng/mL in most children before two years. Thus, we recommend screening for iron overload by serum ferritin when patients receive 5-6 transfusions and consider commencing iron chelation at a lower threshold.

A decade ago, β-thalassaemia was considered a disease with very short life expectancy with significant restrictions to daily activities. This was principally due to the limitations of iron chelator desferrioxamine which is only available as a parenteral preparation that requires 8-12 hour subcutaneous infusions using pumps. However, with the advent of deferasirox, a highly efficacious oral iron chelator, patients with thalassaemia were able to live a near-normal life with minimal restrictions on daily activities. Therefore, it is assumed that these patients have improved quality of life. Contrarily, a recent survey we performed in the three largest thalassaemia centres in Sri Lanka revealed, a large proportion of children with transfusion dependent β-thalassaemia still experience a poor quality of life and have abnormal psychological symptoms [11, 12]. This was more pronounced in the subset of patients with HbE β-thalassaemia. Therefore, it is still important to screen for psychological symptoms and to take remedial steps when caring for patients with β-thalassaemia.

Haematopoietic stem cell transplantation (HSCT) is the only available cure for thalassaemia at present. The best outcome of HSCT with overall survival of 80-90% is reported when transplanting haematopoietic stem cells (HSC) from HLA-matched sibling donors to patients younger than 14-years who do not have significant iron-related complications. However, only about 10% of patients with β-thalassaemia have HLA-matched sibling donors which largely limits the usefulness of HSCT. Additionally, several serious complications related to HSCT that include transplant-related mortality, graft-versus-host disease and graft failure also limits the acceptability and usefulness of the procedure [13].

Gene therapy has been researched over many years as a cure for β-thalassaemia. Contrarily to the initial promise, the progress in the field has been extremely slow. First gene therapy trial for thalassaemia was started in 2007, and thus far over 50 patients have been treated. The

most recent clinical trial demonstrated successful gene therapy in 22 patients with severe β-thalassaemia resulting in reduced need for RBC transfusions without serious adverse events [14]. However, further optimisation is required to improve the efficiency of bone marrow harvesting, transfer of genetic material, level of gene expression and minimise oncogenic potential before gene therapy can be incorporated into standard clinical practice.

Ineffective erythropoiesis due to imbalance between and β-globin chains in erythroid precursors is the primary pathophysiological mechanisms of β-thalassaemia. Growth differentiation factor 11 (GDF11) plays a critical role in this process. Luspatercept, an activin IIB receptor ligand trap which acts in erythroid cells by blocking the interaction of GDF-11 with activin receptors, inhibits ineffective erythropoiesis in thalassaemia. A recent phase 3 trial which evaluated the efficacy of subcutaneous luspatercept in 336 patients with thalassaemia showed >33% reduction of transfusion burden in a subset of patients [15]. With this promising result, luspatercept was recently approved for treatment of adult thalassaemia patients.

Induction of fetal haemoglobin (HbF) by activating γ-globin is another potential therapy for β-thalassaemia. Hydroxyurea, a cytotoxic antimetabolic drug, has produced best results in inducing HbF. Although hydroxyurea increases HbF in patients with sickle cell disease, its efficacy in transfusion dependent β-thalassaemia has not been adequately studied. To evaluate this, we are currently performing a randomised, double-blind placebo-controlled clinical trial in Sri Lanka (SLCTR/2018/24). Additionally, histone deacetylase inhibitor, vorinostat, has shown to be effective in upregulating γ-globin in pre-clinical studies [16].

Genome editing is a new form of gene therapy that recently showed promise in pre-clinical studies of β-thalassaemia. Contrary to traditional gene therapy, genome editing utilises programmable nucleases to create an edit in a pre-determined target site in the human genome. For β-thalassaemia, one approach is to edit genes that suppress γ-globin synthesis, such as *BCL11A*, which is a strong silencer of the γ-globin [17]. Similarly, genome editing of α-globin enhancer has been successfully performed in human HSC to downregulate α-globin, thereby improving globin chain imbalance in β-thalassaemia erythroid cells [18-20]. Both these approaches have shown promise in *in vitro* studies, however, require further validation before progressing into clinical trials.

References

1. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. *Lancet* 2018; **391**(10116): 155-67.
2. Mettananda S, de Silva DG. Anaemia in children: are we using the correct prevention strategies? *Ceylon Med J*. 2017; **62**(2): 73-6.
3. Premawardhana AP, Mudiyanse R, De Silva ST, Jiffry N, Nelumdeniya U, de Silva U, *et al*. A nationwide survey of hospital-based thalassemia patients and standards of care and a preliminary assessment of the national prevention program in Sri Lanka. *PloS one*. 2019; **14**(8): e0220852.
4. Mettananda S, Gibbons RJ, Higgs DR. Understanding alpha-globin gene regulation and implications for the treatment of beta-thalassemia. *Annals of the New York Academy of Sciences* 2016; **1368**(1): 16-24.
5. Mettananda S, Higgs DR. Molecular Basis and Genetic Modifiers of Thalassemia. *Hematology/oncology clinics of North America* 2018; **32**(2): 177-91.
6. Mettananda S. Management of Thalassaemia. *Sri Lanka Journal of Child Health* 2018; **47**(2): 159-65.
7. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. Guidelines for the management of transfusion dependent thalassaemia (TDT) 3rd Edition ed. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. Cyprus: Thalassaemia International Federation; 2014.
8. Fucharoen S, Weatherall DJ. The hemoglobin E thalassemias. *Cold Spring Harbor Perspectives in Medicine* 2012; **2**(8).
9. Mettananda S, Pathiraja H, Peiris R, Wickramarathne N, Bandara D, de Silva U, *et al*. Blood transfusion therapy for beta-thalassemia major and hemoglobin E beta-thalassemia: Adequacy, trends, and determinants in Sri Lanka. *Pediatric Blood & Cancer.* 2019; **66**(5): e27643.
10. Suriapperuma T, Peiris R, Mettananda C, Premawardhena A, Mettananda S. Body iron status of children and adolescents with transfusion dependent beta-thalassaemia: trends of serum ferritin and associations of optimal body iron control. *BMC Res Notes* 2018; **11**(1): 547.
11. Mettananda S, Peiris R, Pathiraja H, Chandradasa M, Bandara D, de Silva U, *et al*. Psychological morbidity among children with transfusion dependent beta-thalassaemia and their parents in Sri Lanka. *PloS one*. 2020; **15**(2): e0228733.
12. Mettananda S, Pathiraja H, Peiris R, Bandara D, de Silva U, Mettananda C, *et al*. Health related quality of life among children with transfusion dependent beta-thalassaemia major and haemoglobin E beta-thalassaemia in Sri Lanka: a case control study. *Health Qual Life Outcomes* 2019; **17**(1): 137.
13. Gaziev J, Marziali M, Isgrò A, Sodani P, Paciaroni K, Gallucci C, *et al*. Bone marrow transplantation for thalassemia from alternative related donors: improved outcomes with a new approach. *Blood* 2013; **122**(15): 2751-6.
14. Thompson AA, Walters MC, Kwiatkowski J, Rasko JEJ, Ribeil JA, Hongeng S, *et al*. Gene Therapy in Patients with Transfusion-Dependent beta-Thalassemia. *The New England Journal of Medicine* 2018; **378**(16): 1479-93.
15. Cappellini MD, Viprakasit V, Taher AT, Georgiev P, Kuo KHM, Coates T, *et al*. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent β-Thalassemia. *New Engl J Med.* 2020; **382**(13): 1219-31.
16. Mettananda S, Yasara N, Fisher CA, Taylor S, Gibbons R, Higgs D. Synergistic silencing of alpha-globin and induction of gamma-globin by histone deacetylase inhibitor, vorinostat as a potential therapy for beta-thalassaemia. *Sci Rep.* 2019; **9**(1): 11649.

17. Canver MC, Smith EC, Sher F, Pinello L, Sanjana NE, Shalem O, *et al*. *BCL11A* enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature* 2015; **527**(7577): 192-7.

18. Mettananda S, Clark K, Fisher CA, Sloane-Stanley JA, Gibbons RJ, Higgs DR. Phenotypic and molecular characterization of a serum-free miniature erythroid differentiation system suitable for high-throughput screening and single-cell assays. *Exp Hematol*. 2018; **60**: 10-20.

19. Mettananda S, Fisher CA, Hay D, Badat M, Quek L, Clark K, *et al*. Editing an alpha-globin enhancer in primary human hematopoietic stem cells as a treatment for beta-thalassemia. *Nature Communications* 2017; **8**(1): 424.

20. Mettananda S, Gibbons RJ, Higgs DR. alpha-Globin as a molecular target in the treatment of beta-thalassemia. *Blood* 2015; **125**(24): 3694-701.

S Mettananda, *Professor in Paediatrics, Department of Paediatrics, University of Kelaniya, Sri Lanka; Consultant Paediatrician, Colombo North Teaching Hospital, Ragama, Sri Lanka.*

Correspondence: SM, *email: <sachith.mettananda@kln.ac.lk>*