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Introductory Chapter: β-Thalassemia

Marwa Zakaria and Tamer Hassan

1. What we need to know about β-thalassemia

Thalassemia is a hereditary, autosomal recessive blood disorder due to partial or complete deficiency in the synthesis of or β-globin chains (β-thalassemia) or α-globin chains (α-thalassemia) that compose the major adult hemoglobin resulting in chronic hemolytic state.

β-Thalassemia is caused by mutations in the HBB gene resulting in decrease of the production of β chain leading to excessive accumulation of unpaired α chains that aggregate and precipitates along the red cell membranes, causing their damage and resulting in intravascular hemolysis. Also, premature destruction of erythroid precursors results in intramedullary death and ineffective erythropoiesis and a short lifespan of mature RBCs in the circulation. Frequent RBC transfusion is the main supportive therapy, leading to excessive accumulation of iron (iron overload), a condition that is exacerbated by excessive hemolysis and the increased iron reabsorption secondary to ineffective erythropoiesis.

Excessive iron is toxic and catalyzes the generation of reactive oxygen species, which in excess are toxic, causing damage to numerous body organs such as the heart and liver as well as the endocrine system. Herein, we represent an overview on thalassemia regarding the underlying pathophysiology of the disease, clinical presentations, and potential therapeutic modalities for the amelioration of its complications, as well as new modalities that may provide a cure for the disease. Thanks to the significant improvement in therapy, patients with β-thalassemia may reach an advanced age.

1.1 β-Thalassemia: types and clinical presentation

In the homozygous state of β-thalassemia which is known as thalassemia major represented with severe, transfusion-dependent anemia within the first 2 years of life. Also associated with skeletal abnormalities and poor growth, in the heterozygous state of β-thalassemia (trait or minor) causes mild to moderate microcytic anemia and not require any specific management. On the other hand, patients in whom clinical severity of the disease lies between that of thalassemia major and thalassemia trait are classified as having β-thalassemia intermedia and require only periodic blood transfusions under special circumstances [1]. Numerous different genotypes are associated with β-thalassemia intermedia, such as HbE which is a common Hb variant found in Southeast Asia, and this variant is included in the beta thalassemia category of diseases. Also, HbS (sickle cell disease) can be presented clinically with severe anemia [2].

Repeated blood transfusions resulting in excessive iron deposition and generation of ROS is a leading cause of morbidity and mortality, in those patients [3].

1.2 Removal of excess iron

Removal of blood (phlebotomy) is used to remove excess iron in patients with normal Hb levels, such as in patients with hereditary hemochromatosis, where iron overload is caused by mutations in the iron homeostasis system [4].

Most other patients with iron overload are anemic (Hb < 10 g/dL) and, therefore, particularly those who are transfusion dependent, phlebotomy would not be optimum and will require iron chelation therapy to decrease iron overload [5].

1.3 Iron chelators

Each unit of transfused red blood cells contains approximately 200 mg of elemental iron. In addition to anemia and ineffective erythropoiesis down-regulates the synthesis of hepcidin, so the use of iron chelators is mandatory to remove excess iron from the plasma as well as from tissues through binding the cheatable, labile, iron form and enhancing its excretion through the body excreta.

Three chelation agents are approved for use in the United States. Deferoxamine, the first to be used, is given by continuous intravenous infusion or slow subcutaneous, infusion through a portable pump. Its main disadvantage is poor compliance secondary to its mode of administration [6].

Deferiprone is an oral iron chelator effective in removing excess iron from the organs and mainly from the heart. The main potential complication is neutropenia that may rarely be followed by agranulocytosis. A liquid formulation has been recently introduced [7].

Deferasirox is available as oral dispersible tablets and is considered as an effective oral chelator, and it reduces liver iron concentration and serum ferritin levels. Deferasirox binds iron with high affinity in a 2:1 ratio. Its main side effects are GIT upsets in the form of abdominal pain, nausea, diarrhea, liver, and kidney dysfunction as well as skin rash. A new formulation of film-coated tablets are now available with better compliance, as it can be taken with a light meal [8].

1.3.1 Shuttle mechanism

The efficacy of chelation may be improved by the use of a combination of chelators. For example, deferiprone may mobilize iron from tissues into the circulation, where deferoxamine binds and facilitates its excretion in the urine this is what is called (the "shuttle mechanism") [9].

1.4 Dyserythropoiesis

Chronic anemia in addition to associated hypoxia in β-thalassemia stimulates excessive RBC production which is mediated through release of erythropoietin, the main erythropoietic stimulating hormone. This attempt is called "stress erythropoiesis" that passes through four steps: expansion of erythroid progenitors, accelerated erythroid differentiation, maturation arrest, and apoptosis. Many other factors, for example, transforming growth factor-β and activin receptor-II trap ligands contribute to this phenomenon. Binding of EPO to its surface receptor on erythroid precursors activates transduction pathways, including Jak2/Stat5, which inhibit apoptosis and stimulates proliferation as well as differentiation of the new cells. However, this operation is futile termed ineffective erythropoiesis due to oxidative stress-increased apoptosis and abortive differentiation [10].

Recent advances in understanding the molecular mechanism involved in two critical steps of dyserythropoiesis are paving the way to new alternative therapeutic targets.

1.5 Novel therapeutic modalities

Several therapeutic modalities aimed at reducing the dyserythropoiesis in thalassemia are currently under research. For example, activin receptor-II trap ligands, JAK2 inhibitors, induction of the Hsp70 chaperone machinery, reducing α-globin synthesis, and stimulation of HbF production [11–15].

1.5.1 Gene modification approach

The hematopoietic stem cells of the affected individual are subjected to gene editing techniques ex vivo and then reinjected again to the patient for reconstitution [16].

To increase the production of γ-globin lentiviral vectors that express a zinc finger protein has been used in order to carry microRNAs that silence its repressors or interacts with the promoter of the γ-globin gene 80 [17].

Genome editing of the promoter of BCL11A can be accomplished by several nucleases, such as engineered zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats linked to Cas9 nucleases (CRISPR-Cas9) (**Figure 1**) [18]. Recently, it has been shown that ZFN-driven BCL11A enhancer ablation leads to increased production of HbF in erythroid progenitors derived hematopoietic stem cell from β-thalassemia patient which could be used for autologous transplantation [19]. Similarly, CRISPR-Cas9-mediated BCL11A enhancer inactivation in a human adult-stage erythroid cell line can achieve the same results [20].
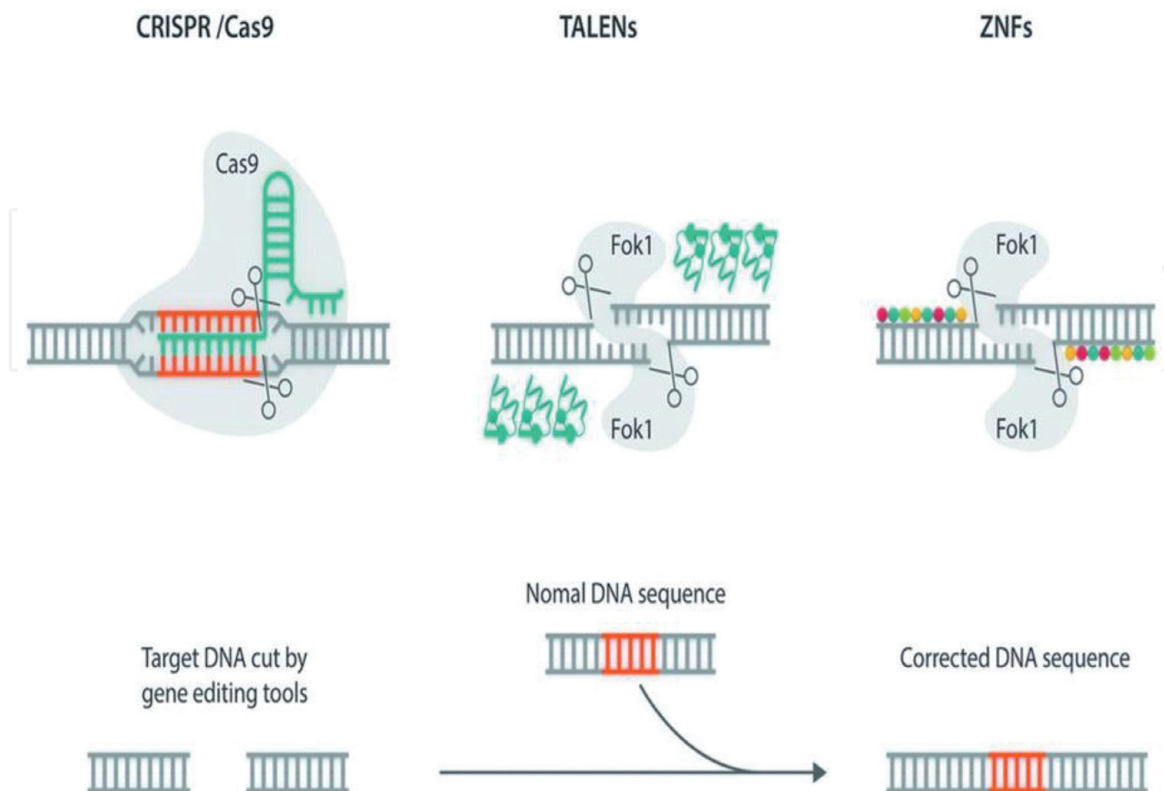

Figure 1.
Mechanism of gene editing.

1.5.2 Gene therapy

Currently, gene therapy represents a novel therapeutic promise, after many years of extensive preclinical research for the optimization of gene transfer regimen. This is mediated through autologous transplantation of genetically modified hematopoietic stem cells, clinical trials being held worldwide have revealed that, by re-establishing effective hemoglobin production, patients may be rendered transfusion- and chelation-independent and escapes the immunological sequel that normally accompany allogeneic hematopoietic stem cell transplantation.

The approach of gene therapy has focused on two mechanisms: first, increasing the production of β-globin by the addition of a normal gene or correction of the mutated gene; and second, increasing the production of γ-globin by the addition of its gene, overexpression of its endogenous activating transcription factors, and silencing of its repressors. Studies of gene therapy have utilized mainly lentivirus vectors in experimental systems, including cultured CD34 HSCs from β-thalassemia patients and β-thalassemia mouse models. Yet the safety profile of such technologies is still uncertain [16].

Genomic editing has been demonstrated to modify the β-globin gene. Thus, TALEN-mediated gene correction has been used in induced HSCs from β-thalassemia patients [21].

1.5.3 Allogeneic hematopoietic stem cell transplantation

Currently, allogeneic stem cell transplant [allo-SCT] remains the only curative option for the majority of patients with β-thalassemia major before development of iron overload complications [22].

Patients with β-thalassemia major who have good risk features are reasonable to anticipate a greater than 90% chance of successful transplant outcome, even patients with high risk features, success rates are approaching 80%.

Challenging in allo-HSCT in high-risk patients is mainly related to graft rejection and risk of transplant-related mortality but nowadays novel modified or reduced-intensity conditioning regimens are used to improve the transplantation outcome in β-thalassemia patients with cheerful results [23].

Traditionally, completely matched human leukocyte antigen identical siblings have been used as donors, and on the other hand, matched unrelated donors have also been tried in patients with low risk. Bone marrow has been known as the preferred choice of stem cells in non-malignant hematological disorders to reduce the risk of GVHD but peripheral blood stem cell graft and cord stem cell when used have been reported to be associated with faster engraftment, lower requirement of blood product support in the peri-transplant period, and a low incidence of graft rejection in patients with low-risk [24, 25].

1.6 Preventive strategies for thalassemia

Despite the great advances in management tools used in β-thalassemia to improve the cure rate of the disease, yet the incidence rate of the disease is increasing especially in underdeveloped countries where the prevalence of consanguineous marriage is high and low level of standard and sometimes shortage in medical resources and supply that limit the early detection of carrier state. Therefore, the prevention of the homozygous state presents a big challenging issue. Prevention

including prenatal diagnosis, carrier detections, molecular diagnosis, and genetic counseling is strongly needed.

1.6.1 Prenatal diagnosis

Recently, prenatal diagnosis is carried out for couples at risk, either in first trimester through obtaining fetal material by chorionic villus sampling or in the second trimester through cordocentesis or amniocentesis.

One of the main successful procedures in prenatal diagnosis is to study the fetal erythroid cells and detection of globin gene mutations. As the first primitive erythroblasts appear in embryonic bloodstream around the 4–5 weeks gestations, so obtaining fetal material by aspiration of coelomic fluid (celocentesis) followed by selection of embryo-fetal erythroid precursors by an anti-CD71 microbeads method or by direct micromanipulator pickup of the cells has been extensively improved and used by several groups [26, 27].

Nowadays, the possibility of cheaper and safer prenatal diagnosis facilities has emerged. Fetal-derived genetic material (cells or cell free DNA) can be obtained from the maternal blood and analyzed, which is considered a non-invasive maneuver with no risk of miscarriage and needs neither complicated procedures nor highly trained personnel for sampling. This allows future screening for thalassemia as well as other genetic diseases [28].

Detection or exclusion of inherited fetal mutations is one of the most important approaches that focuses on detection of mutation that are absent from mother's
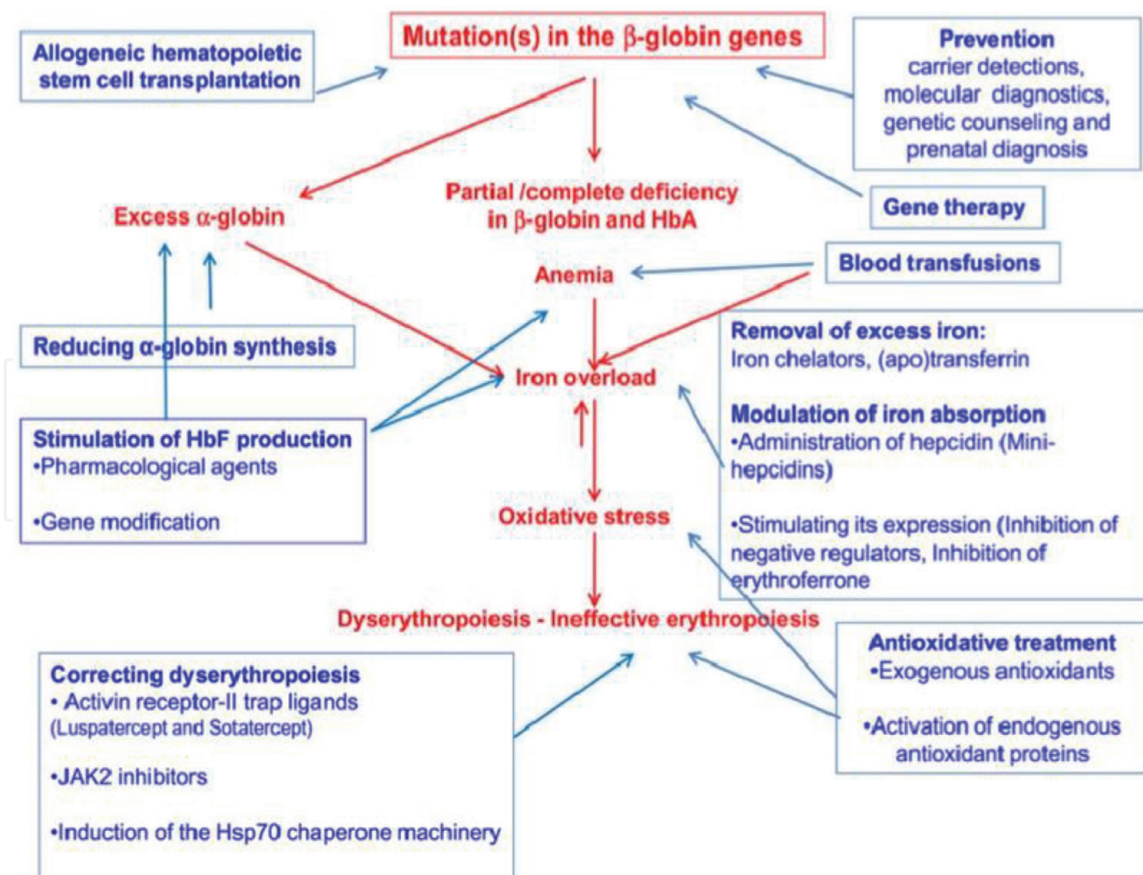

Figure 2.
Beta-thalassemia: causes, symptoms, and therapeutic modalities. Causes and symptoms are marked in red; and therapeutic modalities are marked in blue.

genome that requires DNA quantifications with high sensitivity. Even when the parents have the identical mutation, the relative mutation/haplotype approach might detect this fetal mutation [29].

1.6.2 Pre-implantation genetic diagnosis

One of the promising approaches is pre-implantation genetic diagnosis of cells (PGD) usually single cells, which had been biopsied from oocytes/zygotes or embryos obtained by in vitro fertilization and testing it for specific genetic abnormality. PGD assists couples to avoid birth of an affected child and limit the needs for abortion. This maneuver aims at delivery of an unaffected newborn. PGD helps to identify unaffected embryos for transfer to the uterus [30].

Lastly to conclude, the most likely approach to reduce the patients' load is efficient prevention, carrier detection, prenatal diagnosis, and genetic counseling, and here, we summarize causes, symptoms, and therapeutic modalities in β-thalassemia as illustrated in **Figure 2**.

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