

Transfusion Related Adverse Effects on Beta-Thalassemia Major and New Therapeutic Approaches: A Review Study

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Abstract

Thalassemia is one of the most common genetic disorders, worldwide. Beta-Thalassemia Major (BTM) is the most severe type, which reduces life expectancy and quality of life. In this study, we searched the related keywords to subject from 1996-2019 in the Medline and Web of Science databases, therefore found 250 articles. Moreover, we categorized them into the studies on blood transfusions in beta-thalassemia and new treatment modalities in these patients. We concluded that blood transfusion is the most common therapeutic choice in BTM, but is associated with complications such as iron overload in vital organs. Heart, Liver, Pancreas, Bone Marrow, and Kidney are the main damaged organs. Iron overload related to cardiac dysfunction is the main cause of morbidity and mortality in BTM. Consequently, treatments such as cell therapy and CRISPR/Cas9 are more appropriate compared to the blood transfusion. Cell therapy with lentivirus vectors is one of the novel therapies. The main disadvantages are the differentiation of hematopoietic stem cells into induced Pluripotent Stem Cells (iPSCs), and the human leukocyte antigen mismatch. CRISPR/Cas9 could be used as a promising novel therapy of genetic diseases. CRISPR/Cas9 edits genomes, which is being rapidly grown in clinical use for the former in vivo modification of stem cell-mediated mutations that attempt to edit genes directly in endonuclease encoding. Therefore, in the present study, we described transfusion-related adverse effects in BTM and explained the advantages and disadvantages of new therapies.

Key Words: Beta-thalassemia, CRISPR/Cas9, Hematopoietic stem cell, Lentivirus, Transfusion.

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1- INTRODUCTION

Thalassemia is one of the most common autosomal recessive genetic disorders in the world (1). The disorder is expanded in Sub-Saharan Africa, the Mediterranean, the Middle East, Subcontinent of India, Eastern, and Southeastern Asia. This disorder is initially caused by mutations in the globin chains, Human Beta Globin (*HBB*), and Human Alpha Globin (*HBA*) genes mutations lead to alpha (α) and beta (β) thalassemia, respectively (2, 3). According to the clinical phenotype, beta-thalassemia is classified as minor, intermedia, and major. Minors are usually unaware of their status, and have a normal life, except in challenging statuses like surgery and pregnancy (5). The minor doesn't require a blood transfusion, but intermedia and major required occasionally and regularly lifelong blood transfusion, respectively (1).

BTM signs and symptoms, including jaundice, hypochromic microcytic anemia, are usually observed in 6-24 months of life. However, intermedia thalassemia patients usually have the HB levels >7 and patients stumble between surgery and pregnancy and clinical symptoms occur after 24 months thalassemia becomes intermedia (6-8). The distinction between intermedia and major thalassemia is that

the major is dependent on regular blood transfusion, while there is no need for regular blood transfusion in intermedia (3). Thus, there are some factors to diagnosis these patients in several periods. These indexes include the diagnosis of beta carrier hematological, which are the deduction levels of MCV and MCH. HbA2 is the worth full for b-carrier confirmation (9). Molecular analyses are essential for major, intermedia beta-thalassemia and to distinguish atypical b-thalassemia carrier from perfect a-thalassemia. DNA analysis includes Chorionic Villi to detect prenatal abnormally or numerous carrier screening. In some Islamic territory, hemoglobin screen is compulsory for the couples before getting married (6, 10). Life expectancy in thalassemia patients has increased over the past decades, resulting in the most important milestones in optimizing the management of transfusion and iron colitis (4). Transfusion complications are iron overload, transition infection from contaminated blood bags, which cause failure in vital organs like Heart, Liver, Bone Marrow, Thyroid, Pancreas, and even cause cancer. This is particularly important in pregnancy Patients with thalassemia must be carefully monitoring to avoid iron overload (**Table.1**) (2, 11).

Table-1: Effects of iron overload in patients with BTM and preventive measures (16, 18, and 29).

| Organ | Effects of iron overload | Outcome or finally | Suggests preventive measures |
|-------------|---|------------------------------|---|
| Liver | Hepatic | Cirrhosis | MRI and Evaluation of liver enzymes |
| Pancreas | Abnormal endocrine, abnormalities of glucose metabolism | Diabetes | MRI |
| Bone marrow | Decrease in bone mineral density (BMD) | Osteopenia and osteoporosis | Densitometry should be periodically evaluated |
| Heart | Has a particular negative impact on mitochondria function | Heart failure and myocardial | MRI |
| Thyroid | Hypothyroidism | Thyroid cancer | Annual thyroid monitoring |

2- MATERIALS AND METHODS

To find the related studies, the keywords including "Beta-Thalassemia Major (BTM), Transfusion, Quality of life, CRISPR\Cas9, lentivirus, HSC, iron, and overload" were searched on Medline and Web of Science from 1996-2019. During this period, more than 250 articles were reviewed and the related articles were selected. Then the selected articles were categorized as articles on the effects of blood transfusion on thalassemia major patients and articles on novel therapies for thalassemia.

3- RESULTS

3-1. Bone Mineral Density and BTM

In BTM patients, bone disorders are one of the major causes of the malady. Osteopenia and osteoporosis are the most common beta-thalassemia complications despite regular injections and iron density (12-15). BTM, as a chronic bleeding disorder, results in bone metabolism, which is caused by an imbalance of osteoporosis absorption versus osteoporotic activity (16). The underlying problems of smaller bone size (16) and abnormal bone formation lead to a decrease in Bone Mineral Density (BMD) in patients with thalassemia (17). Diabetes mellitus, hypothyroidism, parathyroid gland dysfunction, accelerated hemorrhage with progressive bone marrow, direct iron poisoning on osteoblasts, effects of iron clotting factors, growth hormone or insulin deficiency such as growth factors and genetic factors cause osteoporosis in thalassemia patients (18). Therefore, there are several explanations on the unpleasant process of bone mineral loss in thalassemia major. Metabolic bone disease is the leading cause of complications in patients with thalassemia major (18). Explanation of the underlying pathogenic mechanisms of bone mineral loss in thalassemia (which is not yet fully understood) is urgent because it allows for optimal treatment and

prevention of these patients. However, considerable disagreement among published reports on the underlying pathophysiological factors of bone mineral loss led to the incompatibility of preventive and therapeutic measures in children, adolescents, and adults with thalassemia major (17). Densitometry should be periodically evaluated in patients with thalassemia to help guide proper treatment at the right time.

3-2. Thyroid and BTM

Changes in thyroid function and thyroid function tests occur in patients with thalassemia major. Physicians caring for patients with TM usually encounter patients with subclinical hypothyroidism in the second decade of life (18). In recent years, our knowledge of hypothyroidism in children, adolescents, and adults with homozygous thalassemia has increased. According to the clinical phenotype of β -thal, its classical classification consists of major (TM), intermedia (TI), and minor (19-22). The thyroid disorders in these patients have different origins. Over 90% of β -thal patients face morbidity and mortality due to transduced iron overload in TDT and increased iron absorption from the gastrointestinal tract in TI (23-25). Thyroid failure usually begins in the second and gradually increases in the third and fourth decades (26). The etiology of thyroid disorders in patients with TM is significantly different compared to the general population. Iron overload transfusion and increased iron uptake from the gastrointestinal tract, thereby affecting erythropoiesis associated with anemia and hypoxia, contribute to over 90% of morbidity and mortality in patients with thal (18, 19, 21, 27). Reversal of subclinical hypothyroidism or improvement of primary hypothyroidism has been observed in some patients with TM who are overloaded after severe iron deficiency. Therefore, periodic evaluation of iron overload and follow-up to improve

adherence to anesthetic treatment and patient satisfaction should be strongly considered to improve the quality of life (QoL), and life expectancy of patients. The emerging issue of thyroid cancer in adult TM patients suggests the need for preventive measures, annual thyroid monitoring in the United States, and careful follow-up (18).

3-3. Diabetes and BTM

The emerging issue of thyroid cancer in adult TM patients suggests the need for preventive measures, annual United State of America (USA) thyroid monitoring, and careful follow-up. The study which was conducted on the fixed subgroup showed that the disorder was the most prevalent in the Middle East (prevalence = 7.90%, 95% CI: 5.75% - 10.05%). The meta-analysis showed that the prevalence of Diabetes Mellitus (DM) in major thalassemia was relatively stable each year. The prevalence of Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance (IGT), and other endocrine abnormalities in beta-thalassemia major was 21.17% (95% CI: 43.8% - 26.00%), 12.46% (95% CI: 5.98% - 18.94%), and 92/43% (95% CI: 37.94% - 49.89%). High prevalence of abnormal endocrine, abnormalities of glucose metabolism was observed in TM (28). Iron overload is most commonly in BTM patients with transfusion therapy. Excess iron during the deposition in the liver, spleen, pancreas, heart, kidney, skin, pituitary, and other organs (**Table.1**) (29).

Endocrine disorders such as diabetes and other diseases in recent years, the prevalence of abnormal glucose metabolism in BTM has gradually increased, which may be associated with increased life expectancy in patients with BTM and increased concern about a combination of Diabetes Thalassemia (30, 31). The most common endocrine disorders were abnormal glucose metabolism, hypogonadotropic hypogonadism, and hypothyroidism.

Patients with thalassemia suffer from the pancreas or other organ dysfunction. In a series of chronic malignant cycles, patients may develop type 1 diabetes due to insufficient insulin secretion or type 2 diabetes due to insulin resistance (32).

3-4. Pregnancy and BTM

Patients should be aware of the potential impact of iron deposition on reproduction capacity, even in cases of donation, with a potential negative impact on the endometrium, resulting in implantation (33). The following aspects must be considered before persuading women with thalassemia (11). Women with thalassemia who wish to become pregnant should have a full evaluation of their iron overload, including T2 and/or SQUID magnetic resonance imaging of the liver and heart. Hemosiderosis, pregnancy should be postponed, and intensification of chelation therapy must be considered (34). Heart function: several factors can compromise heart function during pregnancy including increased blood volume, blood pressure change, heart rate and cardiac output, discontinuation of Chelate therapy, and heart complications are the leading cause of mortality among the patients with thalassemia (35). As a result, all women with pre-pregnancy thalassemia should have a complete evaluation of their cardiac function, including an electrocardiogram (both at rest and under pressure), a 24-hour Holter monitor, a cardiac function evaluated using an echocardiogram, and examined by a Cardiologist (36). Folate demand is typically increased during pregnancy, and all thalassemia women should receive folic acid supplementation to prevent fetal neural tube defects (37). In general, chelatoria is not recommended during pregnancy (38, 39). Women at childbearing age should be warned to refrain from pregnancy or it is advisable to refer to desferrioxamine if any decision is made for pregnancy (40). As expected, serum ferritin increased in most

pregnancies (41-46) due to increased blood intake and discontinuation of the capsule. Risk of specific pregnancy complications, placental ischemic disease, placental abruption, pregnancy hypertension, kidney stones, colitis, and urinary tract infection during pregnancy have been described in women with major thalassemia (43, 44, 47). Thus, threatened abortion and genuine abortion were not more common in women with TM compared to the general population. Otherwise, the consideration of thalassemia as a sign of cesarean delivery remains controversial. The most common cause reported for cesarean is cephalopathy, due to short stature or skeletal abnormalities. In some centers, the policy is to support the patients if they do not decide to have a vaginal delivery factors (44).

3-5. BTM Monitoring

Patients with chronic injections should be evaluated regularly (5). Liver Iron Concentration (LIC) provides an acceptable estimate of total iron in the body. The MRI techniques have generally been replaced with invasive liver biopsy (5). Heart MRI estimation of iron allows accurate prediction of the risk of heart failure and arrhythmia (48). Liver and heart imaging is monitored using the standard MRI, although iron measurement in other organs is under investigation. Serum ferritin should be measured for TDT at least every 3 months, with frequent monitoring of severe chelate adjustment or with ferritin levels below 1000 ng/ml to prevent contraction. Then the cardiac MRI should be monitored annually. Depending on the material used, iron chelator has various side effects. Patients receiving chelation therapy should be regularly selected for their toxicity and laboratory evidence to select the best journal, reduce adherence barriers, and minimize the potential irreversible damages. Iron chalk can cause vision and hearing anomalies, including vision loss, color vision

deficiency, Nyctalopia, Tinnitus, and high-frequency motor hearing loss (49).

3-6. Therapy and New approaches

The purpose of regular RBC transfusion therapy is to relieve symptoms of anemia (allow for normal growth) as well as suppress ineffective endogenous erythropoiesis. This is generally carried out by injecting every 3 to 5 weeks to maintain hemoglobin levels above 9.5 g/d before injection (50). With the increasing use of transfusion therapy, iron overload has become a major problem and chelation therapy is one of the main components of treatment. Chelation therapy is one preventive strategy of iron overload (2).

Recent advances in the genetic treatment of beta-thalassemia (51) include the first human gene therapy trial for beta-thalassemia in 2010 (42) and Human Leukocyte Antigen (HLA) for allogeneic transmission (51). One challenge for the therapeutic use of HSCs induced by iPSCs is the transplantation of these cells to regenerate hematopoietic lines in vivo, although homogenous endothelium derived from hPSCs may have therapeutic potential (52). Clinical trials of beta-globin gene transfer using lentiviruses (LVs) were initiated in the first decade of the new millennium, and recently obtained the results, which not only demonstrate the potential of this method but also suggest better strategies. To maximize therapeutic efficacy. Lens-based gene amplification strategies have been proven to be effective and safe in the treatment of several genetic diseases (53-55). The emergence of LVs and the development of clinical vectors of globin demonstrated the efficacy in the mouse samples (56). Recently, gene therapy (genome editing using the CRISPR/CAS9) has been employed that increases the life expectancy of patients with thalassemia. CRISPR is a revolutionary technology available to researchers to move and edit genomes, which is growing rapidly in the clinical use

for the former in vivo modification of stem cell-mediated mutations that attempt to edit genes directly with endonuclease activity. RNA is a key component of the CRISPR gene deletion system and the CRISPR screen throughout the genome is undergoing routine research, and recent discoveries indicate clinical strength in stem cells (57). Promising future clinical

trials using CRISPR have been presented for treatment ex vivo Genetics in Stem Cells. Development of Hematopoietic Gene Therapy over the past 15 years, immunization, and infiltration have initially involved gamma-viral vectors with several Serious Adverse Events (SAEs), but no SAE has been reported in autologous patients (**Figure. 1**).

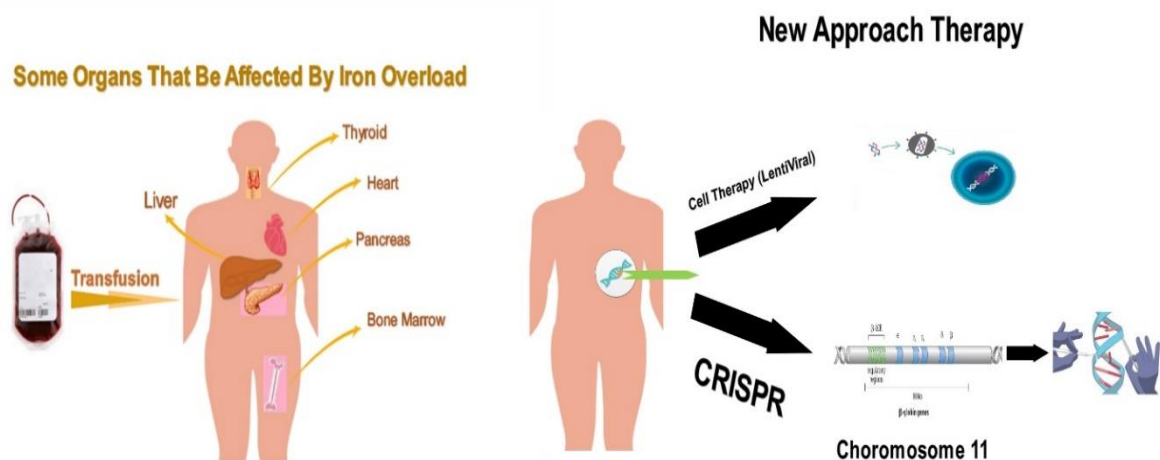


Fig.1: Left: The effects of iron overload on vital organs of the body after blood transfusion Right: in ex vivo: in order to cell therapy need vectors to transfection and after that import to body after transfection. CRISPR need to gRNA and restriction enzyme to cut and correct genome.

New methods of gene editing have been developed to diagnose and treat life-threatening disorders of the hematopoietic system with non-abstract vectors. Genetic repair of autologous HSCs as a result of any type of monogenic disorder with a new therapeutic approach error in inherited hemorrhage becomes new options (58). BTM patients need regular blood transfusions, which support the use of adequate iron density throughout their lives. Many patients with BTM have

limited access to regular and safe blood transfusions, worldwide. The lack of involuntary blood donors, poor awareness of thalassemia, lack of national blood policies, and sporadic blood services create a fundamental gap between timely supply and demand for safety (4). Untreated beta-thalassemia leads to hepatosplenomegaly, bone abnormalities caused by bone marrow expansion, and heart failure due to severe anemia (59, 60) (**Table.2**).

Table-2: Different therapies on beta-thalassemia and its advantages and disadvantages in terms of ease of treatment and impact on patients (1, 52, and 57).

| Type of Therapy | Advantage | Disadvantage |
|-----------------|---------------------------------------|--|
| Transfusion | Increase Life Expectancy | Iron Overload, Transmission of infection |
| Cell Therapy | Don't need transfusion | Laborious · HLA Typing and re-transplanted |
| CRISPR | Gene Editing Increase Quality of Life | Laborious |

4- DISCUSSION

Thalassemia is one of the most common autosomal recessive genetic disorders in the world. BTM is a lifelong severe life-threatening disorder that requires a childhood blood transfusion. Blood transfusion from childhood has several adverse effects like iron overload and subsequently organ damage in youth. Heart, liver, and kidney are the main damaged organs (61). The damages of these organs can significantly decrease the quality of life. Iron overload related to cardiac dysfunction is the main cause of morbidity and mortality among BTM patients. Osteoporosis, chronic bleeding, reduced bone density, and bone deformity are among the symptoms of beta-thalassemia and blood transfusion complications that increase the risk of bone fractures in children (16).

Iron toxicity also affects the growth hormone, growth factor, and affects growth at the childhood ages. According to new studies on the hypothyroidism in beta-thalassemia, the children should be monitored in childhood, adolescence, and adulthood due to the possibility of thyroid cancer (18). Although new iron-chelating therapies significantly decrease the burden of the treatment, this issue remained as the main challenge among patients with BTM. Because of the detrimental effects of blood transfusions on patients, especially children and youth, as they the quality of life, new therapies will be very useful for these patients. Bone marrow

transplantation is a life-saving therapeutic option in BTM, but it may have harmful side effects, because HLA may mismatch between the recipient and the donor. Lentiviral-based therapies have some significant side effects such as bone marrow and liver toxicities (62). Cutting edge technologies like gene therapy and gene editing techniques are the only available curative options (63). Although Lentiviral gene therapy has been widely used, CRISPR/CAS9 gene editing technology changes the overall view of gene therapy, and increase the rate and chance of the treatment (63, 64). Although this technique has an interesting option for the durable therapy of BTM, it is not entered into the clinic. Further investigations on this new gene-editing system can increase the efficiency of this technique (65).

5- CONCLUSION

In conclusion, Blood transfusion has many side effects as well, though transfusion with chelation therapy increased life expectancy. Recent gene therapy is a new approach for this condition and hoped not only to eliminate the need for periodic injections but also to provide sustainable treatment. Although genome-based editing techniques have been proposed in theory, it can revolutionize the treatment of monogenic disorders if implemented. We hope that the new treatments will move from the research phase to the clinical stage and be able to enhance the quality of life for patients.

6- ABBREVIATIONS

BTM: Beta-Thalassemia Major, HBB: Human Beta Globin, HBA: Human Alpha Globin, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, HbA2: Hemoglobin A2, BMD: Bone Mineral Density, TM: Thalassemia Major, TDT: Transfusion Dependent Thalassemia, QoL: Quality of Life, DM: Diabetes Mellitus, IFG: Impaired Fasting Glucose, IGT: Impaired Glucose Tolerance, MRI: Magnetic resonance imaging, SQUID: Superconducting Quantum Interference Device, LIC: Liver Iron Concentration, RBC: Red Blood Cell, HLA: Human Leukocyte Antigen, HSC: Hematopoietic Stem Cell, iPSC: Induced Pluripotent Stem Cell, hPSC: Human Pluripotent Stem Cell, LV: Lentivirus, CRISPR/Cas9: Clustered Regularly Interspaced Short Palindromic Repeats /CRISPR associated protein 9, SAE: Serious Adverse Event.

7- CONFLICT OF INTEREST: None.

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