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Clinical and Molecular Studies on Thalassemia

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ABSTRACT

Thalassemia is a genetic disorder in blood, occurs due to abnormal formation or absent of globin peptide chains of hemoglobin. There are mainly α-globin chains and β-globin chains remain in hemoglobin. When any chain becomes abnormal or dysfunctional then it turns into thalassemia. Defected globin chains are unable to form functional red blood cells, as a result the patients with thalassemia suffer from sever lack of red blood cells as well as available oxygen. The main causes of globin genes disturbance are due to genetic alterations, mainly point mutations. The locations of α-globin genes are at chromosome 16 and β-globin genes are at chromosome 11. β-thalassemia can be divided into three categories, β-thalassemia minor, if one gene is defected; β-thalassemia intermedia, when both β-genes are defected but not at severe level and some chains are functional; and β-thalassemia major, when both globin genes get mutated and globin chains become fully dysfunctional and the patients evolve most of the traits which are responsible for β-thalassemia. Though thalassemia is spread all over the world but every place is not epidemic. Turkey, South Asia, Mediterranean sea area, Iran and some other countries are consider as thalassemia belt. The frequency of thalassemic patients is high at the prevalence zone of malaria because it is considered that thalassemia is against of malaria. The treatments of thalassemia are very complex, expensive and time consuming. However, blood transfusion is more efficient treatment of thalassemia than others. Besides, bone marrow transplantation and gene therapy are the next generation therapies also under consideration.

Key Words: Thalassemia, Anemia, Globin chains, Hemoglobin, Blood transfusion, Bone marrow transplantation

INTRODUCTION

Disease means feeling some disorder or abnormality in the body, caused by different ways like infection, poison, venom, accident, exposures, radiation and genetically from parents to offspring. Some diseases are connected with blood, very sensitive and normally incurable thalassemia is one of them. Thalassemia is an inherited disorder, one type of hemoglobinopathy and causes by abnormal hemoglobin chains. As a result patients suffer from severe lack of functional red blood cells (RBCs). Though the disease thalassemia has been originated more than 6,000 years ago but in 1925, Dr. Thomas Cooley and his colleagues described the disease for the first time (1). The word thalassemia has come from two Greek words 'Thalassae' means 'Blood' and 'Hamia' means 'Sea' and collectively thalassemia means 'Sea in the Blood' as a reference of its prevalence in Mediterranean sea area (2). However, decreased amount of RBCs leads to anemia; as a consequence the oxygen-carrying capacity in blood is reduced. Anemia also destructs the erythroblast in the bone marrow, erythrocytes in the peripheral blood and causes ineffective erythropoiesis (3,4). The proper medication of thalassemia of has not been established yet.

Causes of thalassemia

It is considered that various changes in alpha and beta globin genes are mainly responsible for respective thalassemia. More than 200 mutations (more specifically, point mutations) alter the amino acids compositions, later, turn into abnormal globin peptide chains as a result the shape of hemoglobin becomes changed (5,6). Moreover, inappropriate mRNA splicing, imprecise protein folding also can generate thalassemia at any stage of life. Furthermore, if one or more globin genes become dysfunctional or mutated then at least there is a chance for a person to be a carrier of thalassemia (Table 1).

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Thalassemia prevents malaria

In 1945, J.B.S Haldane observed that the frequency of thalassemia is high at the prevalence areas of malaria. Nevertheless, at those belts the thalassemic carriers were surviving more than non-thalassemic persons (7). Haldane hypothesized that the mutations in globin genes were somehow prevented the deadly case of malaria though the mechanism was not clear. That protection was randomly and the parasites of malaria could not complete their life cycle in abnormal red blood cell.

Table 1: Different genetic changes which are responsible for thalassemia

Gene	Location	Change	Consequence
β-globin	Codon 17, 39, 43	CAG → TAG (Nonsense mutation)	Glutamine to stop codon (8,9)
β-globin	first intervening sequence (IVS-1)	GTC → ATC (Point mutation)	Threonine to Proline (10)
β-globin	Codon 06	GTG → G_G (Thymine Deletion)	Change in frame shift (11)
β-globin	Codon 01 Codon 76	GTG→ _TG, (Guanine deletion) GCT→ G_T (Cytosine Deletion)	Change in frame shift (12)
β-globin	Within codons 41 and 42	-CTTT (four nucleotides deletion from the gene)	Frame shift deletion (13)
β-globin	3' end of intron 01	G→ A (substitution in intron 01)	Error in RNA splicing (14)
β-globin	At position -29 nucleotide	A→ G (Single base substitution in TATA box)	Abolish mRNA splicing (15)
β-globin	At position -101 nucleotide	C→T (transverse mutation)	Silent carrier (16)
β-globin	first intervening sequence (IVS-2)	Transposable element insertion in the β-globin gene	Gene inactivation (17)
Erythroid transcription factor	X chromosome	Mutations in the transcription factor GATA1	β-thalassemia by thrombocytopenia (18)

*[**Note:** Here, '_' indicates deletion of a single nucleotide]*

Transmission probability of thalassemia from parents to children

When both parents are healthy means do not have any mutation or defect in globin genes then there is almost no chance to transmission of thalassemia from parents to children. If one of parents is either affected or carrier, then there is a chance for children of being thalassemia carriers. However, when both of parents are carriers then according to the first law of mendel there are 25% chance for a child to be totally healthy, 50% chance to be a carrier and 25% chance to be a patient of thalassemia (Figure 1).

Epidemic zone of thalassemia

The prevalent area of this disorder is known as Thalassemic Belt. Iran is a high prevalent zone of thalassemia. Alpha thalassemia commonly found in the South Asia and in Southern part of China. However, Beta thalassemia is widely spread in the world commonly found in the Mediterranean sea area, the Middle East, the mainly the Indian subcontinent, Russia and Northern part of China (5,7,19). Previously thalassemia was known as one of the most common inhered Hemolytic Anemia in South East Asia (20, 21).

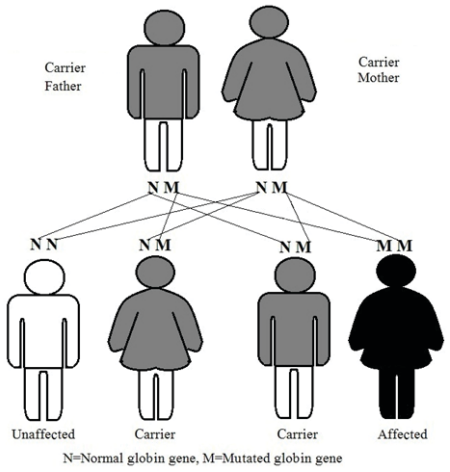

Figure 1: The probability of thalassemia sagrigation from carrier parents to children (figure was illustreted by author).

[Note. Here, NN=Healthy (white), NM=Thalassemia carrier (gray) and MM=Thalassemic patient (black).]

Types of thalassemias

Thalassemias are heterogeneous group of Mendelian disorders, which are characterized by the abnormal synthesis of α or β-globin chain of hemoglobin. Thalassemia also can be

classified according to the modicum in the globin chain formation. There are four hemoglobin chains which are divided under two main types are Alpha (α) chains and Beta (β) chains. There are also some auxiliary chains associated with alpha and beta chains are known as gamma (γ), delta (δ), zeta (ζ), epsilon (ε) (20). These auxiliary chains are also responsible for thalassemia. There are four genes which responsible for formation of α-globin chains and two genes for β-globin chains. When one or more genes related with α-globin chains become defective or mutated then it causes α-thalassemia. Same as, defective genes for β-globin chains are responsible for β-thalassemia.

1. Alpha thalassemia

Alpha thalassemia is a genetically autosomal recessive defect caused by decrease production or total absence of alpha globin peptides due to mutation in associated genes. Mainly deletions of nucleotides occur in one or more of four alpha globin genes. The respective genes of alpha globin are present in chromosome number 16. The normal feature of α-globin gene is indicated as αα and its genotype is indicated as αα/ αα. When mutation occurs in one or both globin genes then it is mentioned as "_ α" or "_ _" respectively. These also represent the most common forms of α-thalassemia. It is more common in South Asia than any other zone of the world (22).

Types of α–thalassemia

Clinically, both forms of deletional and nondeletional α-thalassemia can be broadly classified into four categories depending on the number of functional α-globin genes.

***Type-1*:** When four genes are mutated then following category is known as Haemoglobin (Hb) Bart's hydrops or α-thalassemia major (_ _ /_ _) (22,23).

***Type-2*:** When there is inheritance of just one functional α-globin gene out of four genes (_ _/ _ α) then it is known as Hb H disease or α-thalassemia intermedia. Patients with Hb H due to nondeletional types show chronic haemolytic anaemia of severity than with deletional type (23,24).

***Type-3*:** If two genes of α-globin are infected or mutated in *cis* or *trans* position so the talassaemia is called α-thalassemia trait or α-thalassemia minor, results in heterozygousity for α0 thalassemia (_ _/ αα) and homozygosity for α+ thalassemia (_α /_ α) (23).

***Type-4*:** When three genes of α-globin chain are functional and only one gene is defected or dysfunctional (_ α/ αα) then it syndromes as α-thalassemia silent carriers and exhibit no clinical abnormality (23). *(**Note:** Here '_' indicates mutation in the α-globin gene)*

2. Beta thalassemia

In general, most of the thalassemia is descendent from parents to children as autosomal recessive traits. Beta (β) thalassemia is originated by a group of genetic disorders of β-globin genes, present in chromosome number 11. Mutations in β-globin genes lead to either decreased amount or completely absent of β-globin polypeptide which results abnormal hemoglobin (Hb) in red blood cell (RBC) consequently turns into Anemia (25).

Symptoms of β-thalassemia

Phenotypically β-thalassemia is known by several signs and syndromes. If a patient carries these symptoms, then a physician preliminary can determine that the patient may have β-thalassemia. These are: anemia variable severity, dark urine, enlarged abdomen, liver, spleen, feeding problems (infant), susceptibility to infection, jaundice and so on (26).

Types of beta thalassemia

There are three main classes of beta-thalassemia based on disease acuity, β-thalassemia minor, intermedia and major.

i) Beta-thalassemia minor

If a single gene of β-globin chains becomes mutated of dysfunctional any how then thalassemia minor originated silently. The patients of minor β-thalassemia may not expose any symptom during the disease except occasional reduced anemia and sometimes patients may require blood transfusion. It may be inherited when both parents carry a single mutated gene of β-globin and risk at every child will remain under 25% risk of homozygous β-thalassemia (26,27).

ii) Beta-thalassemia intermedia

Beta-thalassemia intermedia is the middle phase/condition according to the severity of thalassemia disorder. Here, one or both β-globin genes may be mutated but the β-globin chains will not be totally present or absent and after a certain time it turns into β-thalassemia major. The patients of β-thalassemia intermedia generally suffer from defects in different bones, deformities of face, osteoporosis, ulcer in legs, increased thrombosis, stroke, pulmonary embolism and so on (26,28**)**. Moreover, iron overload in intestine and hypogonadism effect are other alarming concerns. The patients require frequent blood transfusion and regular medication (29).

iii) Beta-thalassemia major

Beta thalassemia major occurs when both β-globin genes become mutated or fully unable to function; as a result β-globin chains remain absent in hemoglobin. If the patients of β-thalassemia major remain poorly transfused or untreated

then the clinical features of β-thalassemia major are evolved. A patient who is suffering from severe β-thalassemia major, usually bears a lot of physical and morphological changes in various organs of his/her body. Mainly size and shape of liver and spleen become abnormal, deformities in the femurs, typical craniofacial changes in the skull, depression of bridge in the nose, some changes in the teeth placement also can be found among the patients (28). The patients need regular blood transfusion or successful bone marrow transplantation and regular observation by experience physicians and staffs.

TREATMENTS FOR THALASSEMIA

The patients are affected with thalassemia, considered 'Half-Related Quality Of Life (HRQOL). The complete medication or curing has not been established against thalassemia. However, there are some supporting treatments which are referred and applied to the affected patients to reduce the severity of the disease. The most potential treatments are:

i) Blood transfusion therapy

Regular blood transfusion is the most common treatment of thalassemia intermediated and major. The routine of blood transfusion and amount of blood depends on the severity of the disease; however, it may be once a month or two months or six months. The most concern of blood transfusion is the quality and homogeneity of donor blood (30). The blood must bears some criteria such as, donor and recipient blood group and rhesus must be same, diseases and contamination free, allergy free, pathogens screened, and blood components complying with standard guidelines by the National Blood Centre. In this case, nursing staffs must bear high experiences and the patients, receiving blood transfusion need regular quality care.

ii) Bone marrow transplantation (BMT)

Blood is produced in bone marrow which contents some hematopoietic stem cells having a capacity to form different blood cell components. If the bone marrow of a healthy donor can transplant to a thalassemic patients properly then there is a chance to recovery normal RBCs production by the patient *in vivo*. The first successful BMT of β-thalassemia was in 1982 (31). A successful BMT therapy can lead to a success of β-thalassemia free survival significantly but the main challenge of BMT is adjustment of donor bone marrow with recipient. In general, our body system either reject any foreign elements directly of makes a antigen antibody reaction. In the case of BMT, it is subjected to finding a donor with an identical Major Histocompatibility Complex (MHC). Homologous linage is another important issue, as donors healthy parent, sibling and near relative are better choice (26,32).

iii) Hydroxyurea therapy

Lack of functional globin chains can be back up by enhancing γ-globin chains synthesis. However, the increased number of γ-globin chains reduce α and/or β-globin chains imbalance; potentially, γ-globin leads to progress RBCs. Hydroxyurea is a pharmacologic agent that increases γ-globin production and boost up fetal hemoglobin (HbF) (33). So it is considered as a alternative therapy for patients, suffering from β-thalassemia. The considerable advantage of increased expression of γ-globin gene is that it regulates the production of excess α-chain (34).

iv) Gene therapy

The gene therapy has been a next generation concept to cure thalassemia, though the success rate is not significant till now. It focused on utilizing retroviral vectors to insert desire globin genes into the target cell, so that the genes become capable to integrate with host cell genome precisely. The expectation of gene therapy is to avoid abnormal gene expression or silencing but long term normal gene expression orderly. Nevertheless, to be an effective and realistic therapeutic approach gene needs to meet the following criteria: a) donor and recipient should have lineage specificity; b) the therapeutic vector should exhibit stability; c) gene of interest must have respective regulatory elements; d) proper trans-gene expression and precise localization at sustainable levels; e) the therapeutic process must be safe and contamination free and so forth (35).

DISCUSSION

The diseases which are not linked with permanent genetic changes are fully curable by proper medication, care and exercise. But the diseases are descendent from parents to offspring genetically are not easy to think to cure properly. Thalassemia is an inherited autosomal recessive disorder; it passes from generation to generation by vertical gene transformation. It is not possible to remove the disease from the patients permanently. However, the disease can be controlled by some managements and regular practices.

To prevent thalassemia there is another option at prenatal stage. The parents should have definite diagnosis and appropriate counseling on their health condition. Everyone should check him/herself that either he/she is a thalassemia carrier or not. If both of couple are carriers then they need counseling regarding their chance of having an affected child. There are some genetic markers which can differentiate among affected patients, carriers and unaffected healthy.

Nevertheless, besides blood transfusion it is essential to develop next generation novel drugs for the patients who are suffering from thalassemia and its side effects. Artificial hemoglobin or RBCs production and successful combination with blood stream may be a breakthrough in the treatments

of thalassemia. Moreover, chelating agent preparation is also important which can capture free iron and reduce iron overload in the blood of the patients.

The patients who do not get proper and sufficient treatment can suffer from several complications, such as increased size of spleen, heart failure, frequently clot formation inside blood vessels, high susceptibility microbial infection growth failure, endocrine dysfunction, delayed sexual maturity and so on.

CONCLUSION

Thalassemia is a very serious inherited disorder present among hundreds of thousands of people. It starts from beginning of the life and the frequency of the disease can be observed among infants and babies because the patients do not exist longer. Though it is considered that several mutations are the main causes of thalassemia but most of the cases it descendents from ancestor to new progeny. Affected or carrier parents may give birth a affect baby or a carrier. It is a matter of sorrow that no proper treatment has been developed yet to cure thalassemia fully, it can control only for some times. Though there are some treatments existing but not free from side effects or limitations, moreover, the treatments are very expensive and time consuming. It is matter of hope that thousand of scholars and scientists are trying to remove the disease by inventing novel drugs. Gene therapy, bone marrow transplantation and induced pluripotent stem therapy are also going on besides blood transfusion. Prenatal diagnosis and counseling can lessen the frequency of affected new born babies. To make worldwide awareness about thalassemia the World Health Organization (WHO) has declared 8th May as international thalassemia day since 1994.

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ABBREVIATIONS

1. A: Adenine

2. C: Cytosine

3: G: Guanine

4: T: Thymine

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