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REVIEW

The Importance of IRP as Regulators of Iron Metabolism in β Thalassemia Patients

Yasemin KARTAL[1,2], Dilek KAYA-AKYÜZLÜ[1], Zeliha KAYAALTI[1]

ABSTRACT

Thalassemia is a hereditary blood disease. Beta (ß)-thalassemia which caused by a point mutation on ß-globin gene localized on short arm of chromosome 11 as a cluster, is an autosomal recessive and also one of the most common genetic diseases in worldwide. In other words, this disease is characterized by malfunctions during the globin chain synthesis of hemoglobin synthesis process. Unbalanced globin chain synthesis is the major cause of low level hemoglobin production leading to anemia. Current therapy for this disease includes regular blood transfusions and iron chelation. Excessive hemolysis, increased intestinal iron absorption as well as frequent blood transfusions during therapy results in chronically increased iron load and consequently oxidative stress. Iron homeostasis is provided by iron regulatory proteins (IRPs) in body. Therefore, variants of these proteins lead to differences in iron uptake and the storage. In this review, the role of IRP-1 and IRP-2 in iron uptake and the effect of oxidative stress consequent upon the iron toxicity in thalassemia individuals will be examined in detail.

Keywords: IRP-1, IRP-2, gene polymorphism, oxidative stress and thalassemia.

Overall View on Thalassemia

Thalassemia term is derived from thalassa (sea) and haima (blood). According to the literature, thalassemia is not only restricted to the Mediterranean countries, but it has also emerged in tropical countries covering a wide area. It is one of the most common autosomal recessive disorders worldwide and this disease is often associated with inadequate synthesis or no synthesis of the globin chains. The mutations occur in β-globin gene causes β thalassemia and abnormal hemoglobin formation (1). β-globin chain production mechanism is defective in patients with beta-thalassemia. In contrast, α-globin chain synthesis continues normally due to the fact that α gene in these patients is not affected. However, α-globin chains can not combine with β-globin chains due to its excessive amounts and consequently hemoglobin tetramers could not form. Blood transfusions are required at regular intervals for treatment. As a drawback, iron load is increased as a combined result of excessive hemolysis, increased intestinal iron absorption as well as frequent blood transfusions. Foretold progressive iron overload has become a major cause of organ damage and organ dysfunction (2, 3).

In recent years, there has been an important advancement

Yasemin Kartal
[2]Hacettepe University, Faculty of Medicine, Department of Physiology, Ankara, TURKEY

Dilek Kaya-Akyüzlü, Zeliha Kayaaltı
1Ankara University, Institute of Forensic Sciences, Ankara, TURKEY

Corresponding Author
Zeliha Kayaaltı
Tel.: +90 312 3192734
Fax: +90 312 3192077
E-mail: kayaalti@ankara.edu.tr

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in knowledge regarding iron metabolism regulation which can provide us better understanding of the pathophysiology of beta thalassemia. Iron homeostasis is regulated by iron regulatory protein (IRP).

Iron Regulatory Protein (IRP)

Iron, plays an important role in many organisms, is the prosthetic group of proteins involved in cellular processes such as oxygen transport and DNA synthesis. While low cellular iron levels results in deterioration of the cellular proliferation, high cellular iron levels catalyze the production of free radicals which eventually damage proteins, lipids and DNA. For this reason, the regulator mechanisms for iron uptake, storage and transportation must be seamless in order to maintain normal cellular iron content.

IRPs play important roles in cellular iron homeostasis due to post-transcriptional regulation of several iron metabolism proteins related with the uptake, transport and storage of iron via binding to the iron responsive element (IRE) located on UTR of mRNAs encoding (4, 5). IRPs also regulate both 5' IREs of mRNA comprising mitochondrial aconitase, erythroid aminolevulinat synthase (eALAS), hypoxia inducible transcription factor-2α, myloid β precursor protein and also 3 'IRE of mRNA comprising divalent metal transporter 1 (DMT1), CDC14 (mitotic phosphatase), Cdc42-dependent protein kinase α and hydroxyacid oxidase 1 (6–15). Recently, transcriptome approach was used to determine mRNAs containing 35 new IRE which are specific to IRPs (16).

There are two IRPs in mammalian cells including IRP-1 and IRP-2 which are regulated by different mechanisms. While IRP-1 are highly expressed in the brown fat, kidneys and liver, IRP-2 are highly expressed in the central nervous system (17).

IRPs sense cytosolic iron levels and alter their activities according to intracellular iron levels (18). Considering this situation in more detail, when cells are in excess iron, IRP-1 can not bind to 5' IREs of ferritin mRNA and results in an eventual increase in the synthesis of ferritin. In contrast, when cells are in iron deficiency, IRP-1 binds to 5 'IREs of ferritin mRNA with high affinity to suppress translation of ferritin.

Considering the exact opposite situation, when cells are in excess iron, IRP-2 can not bind to 3' IREs of transferrin receptor 1 (TfR1) mRNA. As a result of this, the mRNA's stability is deteriorated with the endonuclease activity and eventually synthesis of TfR1 is decreased. In contrast, when cells are in iron deficiency, IRP-2 binds to 3' IREs of TfR1 mRNA and eventually increases the synthesis of TfR1 (19-21).

In β thalassemia patient, iron load is increased as a combined result of excessive hemolysis, frequent blood transfusions and increased intestinal iron absorption. Therefore, variants of IRPs lead to differences in iron metabolism in β thalassemia. In addition, polymorphism of IRP-1 and IRP-2 alter metal toxicokinetics in patients with thalassemia and effect both blood toxic metal and trace element levels.

Hereditary hemochromatosis and cellular accumulation of iron also leads to cirrhosis, cardiomyopathy and diabetes mellitus (22). Excess iron accumulation in the brain is associated with numerous common neurodegenerative diseases such as Alzheimer, Parkinson as well as it is found to be associated with inherited neurodegenerative disease like Friedreich's ataxia (FA) (22-24). On the other hand, iron deficiency affects millions of people worldwide due to anemia in adults and cognitive damage in children. Therefore, cellular iron content must be contained within a narrow range in order to avoid the negative effects of its excess presence or deficiency.

Systemic Iron Metabolism

Total body iron is about 4 grams (3-5g) in healthy adult (25). These 4 grams are distributed throughout the body in hemoglobin, bone marrow, ferritin, hemosiderin, myoglobin, transferrin, cytochromes and iron-enzyme complex (26).

Although iron can be absorbed from every part of the gastrointestinal tract, maximum absorption is present in the upper part of duodenum and jejenum. Thus, absorption rate of iron steadily declines as it goes to the lower parts of the intestines (25). In addition, dietary non-heme ferric iron is absorbed by enterocytes with the help of DMT1. Because of the reason that Fe^{+3} iron is not water soluble and must be converted to a water-soluble structure to be absorbed, firstly ferric iron (Fe^{+3}) is converted to ferrous iron (Fe^{+2}) by membrane bound ferrireductases (DCYTB). After the absorption phase, iron is either stored as ferritin or transported to circulation by ferroportin which is a basolateral transporter. Ferroportin is essential for iron export from hepatocyte, macrophages and enterocytes (27). Iron transport depends on the oxidation by membrane bound multicopper oxidase hephaestin. Iron is converted to Fe^{+3} form by hephaestin for binding to plasma transferrin (Tf).

Transferrin plays major role in iron metabolism. Because of the reason that, iron is carried with transferrin in the plasma

and it is given to tissues in times of need. After diferrik Tf (Tf-Fe (III)) binds to TfR1 which resides on the cell surface, Tf-Fe(III)–TfR1 complex is taken into the cell by clathrin-mediated endocytosis. Then, ferric iron release from Tf is stimulated by endosomal acidification (pH below 5.5). Ferric iron is reduced by STEAP3 which is a metalloreductase and then it is transported into the cytoplasm by DMT1. After endosomal acidification, apotransferrin which is now in non-ferrous state has a high affinity for the transferrin receptor. Apotransferrin-receptor complex is then transferred back to the cell surface by endocytosis mechanisms. Apotransferrin loses affinity for the receptor by contact with a neutral pH on the cell surface. Thus receptor surface becomes suitable for reuse (28, 29).

Mitochondria have a particularly important role in iron metabolism. Because cytosolic iron is used by the mitochondria to form protein containing iron (Fe-S clusters) as well as used in various critical steps in heme formation that takes place in the mitochondrial matrix where ferrochelatase and 5-aminolevulinate synthase (ALAS) are present (30-32).

Iron Toxicity

Iron is an essential mineral for normal cellular physiology, but excess amount can result in several pathological conditions such as cancers, liver and heart disease, hormonal abnormalities and immune system dysfunctions. Iron toxicity is largely based on Fenton and Haber–Weiss chemistry which damage cellular macromolecules, promote cell death and tissue injury. In Fenton and Haber–Weiss chemistry, hydrogen peroxide (H_2O_2) formed by normal cellular process can react with iron. This reaction turns Fe^{+2} into Fe^{+3} form and free hydroxyl radical ($OH\cdot$) is also formed as by product (Figure1). Consequently, these products lead to the oxidation of proteins, lipid peroxidation in organelles such as lysosomes and mitochondria, modifications of the nucleic acid and progressive parenchymal cell damage. In addition, large amount of iron present in the cells leads to some defects in structure of transferrin, ferritin and other iron binding proteins (33, 34).

The Fenton reaction:

$$Fe^{+2} + \boxed{H_2O_2} \longrightarrow Fe^{+3} + \boxed{OH\cdot} + OH^-$$

The Haber-Weiss reaction:

$$\boxed{O_2^-} + \boxed{H_2O_2} \longrightarrow O_2 + H_2O + \boxed{OH\cdot}$$

Figure1. Haber–Weiss chemistry.

β thalassemia patients are mainly exposed to oxidative stress due to iron overload which is the joint outcome of multiple blood transfusions and inappropriately increased iron absorption associated with ineffective erythropoiesis (35). Iron overload leads to transferrin saturation, eventually increasing the circulating free iron concentration (termed as non-transferrin-bound iron) which catalyzes the formation of free radicals, resulting in oxidative stress (35, 36). In addition, increased α globin chain also causes a high degree of oxidative damage in β thalassemia patients and it changes in the antigenic structure of erythrocyte membrane (37, 38). For this reason, β thalassemia patients are in a state of enhanced oxidative stress.

The State of The Oxidant Balance

Increase of free radicals cause oxidative stress which results in metabolic damage in biological macromolecules. Multifactorial mechanisms facilitate oxidative damage in β thalassemia patients because of the fact that free, unpaired, unstable globin subunits create superoxide and hydroxyl radicals which lead to protein aggregation and hydroxylation of DNA. In addition, hydroxyl radicals lead to decrease in the deformability with membrane skeleton impairment, peroxidation of membrane lipids, increase in rigidity and loss of intracellular K^+ ion by deterioration of cation exchange (39).

Iron overload and pro-oxidant/ anti-oxidant imbalance progressively affect β thalassemia patients. For example, many studies have shown that lipid soluble and exogenous antioxidants such as vitamin E (α-tocopherol), vitamin A (retinol), β-carotene were lower in β thalassemia (βT) patients than in controls (40-43). Similarly, reactive thiol groups found to be decreased in the plasma (44).

Kalpravidh *et al.,* found that Coenzyme Q10 (ubiquinone) levels which provides antioxidant protection in the body and functions as an effective scavenger of free radicals were decreased in βT patients (45).

In addition, studies on trace elements such as selenium (Se), copper (Cu), zinc (Zn), magnesium (Mg), and calcium (Ca) reveal significant change in plasma concentration of these elements in thalassemia patients. In these patients, Se level which is an essential constituent of the enzyme glutathione peroxidase was significantly decreased when compared to the controls (46). This decline could be explained by overload of iron in patients that possibly decrease Se absorption from

intestinal tract (47).

Cu which both is the major component of hemoglobin and is a central component of the antioxidant superoxide dismutase molecule was found to be significantly increased in βT patients when compared with controls. The antagonistic effect of the Zn could explain the increased level of Cu (48).

Zn which has numerous significant antioxidant properties and protects cells from damage due to free radicals was found to be significantly decreased in βT patients when compared with controls. The decreased level of Zn could be explained by iron overload that possibly decrease Zn absorption from intestinal tract (47).

Superoxide Dismutase (SOD), an essential antioxidant, protects the cells from damage via decreasing the formation of reactive oxygen species. Carpino *et al.,* pointed out that SOD activity was decreased in patients with thalassemia as compared to controls (49). In parallel, Dhawan *et al.,* are in agreement with results about decreasing SOD activity in βT patients when compared with controls (50).

Catalase (CAT), an intracellular enzyme, is responsible for detoxification of H_2O_2 in the cells via decomposing H_2O_2 into water and oxygen molecules. Attia *et al.,* 2011 showed that serum catalase levels in βT patients were lower than in healthy controls (51). This result is similar to Mahdi in 2014 pointing out that CAT levels were significantly lower in βT patients as compared with healthy controls (46). The decreased level could be explained by increasing MDA which is a product of lipid peroxidation (52).

Glutathione (GSH) has a vital role in protection of erythrocytes and leukocytes against oxidative stress due to a major intracellular reducing agent. According to previous study, GSH levels were significantly 2-3 times lower in βT patients as compared with healthy controls (46).

Glutathion peroxidase (GPX) is the most effective antioxidant against oxidative stress by catalyzing the reduction of lipid hydroperoxides. It is reported that GPX levels were significantly lower in βT patients as compared with healthy control (46). The decreased level could be explained by increased super oxide anion which eventually inactivates GPX (53). In parallel, some studies showed that glutathione reductase levels were 3 times lower than in healthy controls (54).

Iron is a major free radical creator and leads to oxidative damage by providing conversion of hydrogen peroxide to free radicals. Its metabolism needs to be tightly controlled due to the fact that either iron deficiency or iron excess can have adverse consequences on organ function and tissue integrity (55, 56). Iron and iron-related oxidative stress play an important role in the pathogenesis of various diseases such as thalassemia and age-related macular degeneration (AMD). Synowiec *et al.,* in 2012 pointed out that polymorphisms associated with iron metabolism affect individual susceptibility to AMD in which both oxidative stress and genetic factors in combine to play an important role in the pathogenesis of the disease (57). Results of several studies have shown AMD may be exacerbated by retinal iron and the concentration of retinal iron increases with age which may result in cumulative oxidative damage because of ROS (55, 59).

Synowiec *et al.,* 2012 stated that genetic polymorphism in genes related to iron metabolism may predispose individuals to the development of AMD and therefore they checked for an association between rs867469 in IRP1 and rs17483548 in IRP2 (57). So, genetic polymorphism in genes related to iron metabolism might be crucial for patients with β thalassemia.

Conclusion

In conclusion, iron is an important source of ROS generation despite its' role as an essential element which plays a crucial role in electron transfer and catalysis. IRP regulates translation of proteins related with the uptake, transport and storage of iron. So, polymorphism in genes related to iron metabolism can affect exposure of individuals to iron toxicity. Iron overload that identified in thalassemic patients' blood and tissues is responsible for the generation of oxidative stress which plays an important role in many pathophysiological processes. As foretold by numerous studies, plasma concentration of trace elements and antioxidant enzymes found to be significantly different in thalassemia patients compared with healthy individuals. Therefore, it is a rationale to support iron chelation therapy for the elimination of the free-iron species and to promote the free-radical scavenging activity of the antioxidants.

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β Talasemi Hastalarında, Demir Metabolizması Düzenleyicisi olarak IRP'nin Önemi

ÖZ

Talasemi, kalıtsal kan hastalıkları içerisinde yer almaktadır. Dünyada en yaygın genetik hastalıklardan biri olan Beta (ß)-talasemi, 11. kromozomun kısa kolunda küme olarak lokalize olan ß-globin genindeki nokta mutasyonların neden olduğu otozomal resesif bir hastalıktır. Diğer bir deyişle, hemoglobin molekülünün üretimi süresince globin zincir sentezlerindeki düzenlenme dengesizliklerine dayalı globin zincir sentez bozukluğudur. Dengesiz, globin zincir sentezi düşük hemoglobin üretiminin başlıca nedenidir ve anemiye neden olmaktadır. Günümüzde uygulanan tedavi yöntemi regüler kan transfüzyonları ve demir şelasyonudur. Talasemik olgularda gerek artmış hemoliz, gerekse intestinal demirin fazla absorbe edilmesi ayrıca uygulanan sık transfüzyonlar sonucunda vücut demir yükü kronik olarak artmakta ve bu olgularda ortaya çıkan aşırı demir birikimi oksidatif strese neden olmaktadır. Demir homeostazı demir düzenleyen proteinlerle (IRPs) sağlanmaktadır. Bu nedenle, bu proteinlerin varyantları demir alımı ve depolanmasında farklılıklara yol açacaktır. Bu çalışmada, IRP-1 ve IRP-2'nin demir alımındaki rolü ve demir toksitesine bağlı gelişen oksidatif stresin talasemili bireylerdeki etkisi irdelenecektir.

Anahtar kelimeler: IRP-1, IRP-2, gen polimorfizmi, oksidatif stres ve talasemi.

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