

*Review Article***EFFECT OF IRON OVERLOAD CARDIOMYOPATHY IN HAEMOCHROMATOSIS AND  $\beta$ -THALASSEMIA****Sairah Hafeez Kamran<sup>1</sup>, Uzma Saleem<sup>2</sup>, Bashir Ahmad<sup>1</sup>, Mobasher Ahmad<sup>1</sup>**<sup>1</sup> University College of Pharmacy, University of the Punjab, Lahore 54000, Pakistan<sup>2</sup> College of pharmacy, Government College University, Faisalabad, Pakistan**ABSTRACT**

Iron overload cardiomyopathy is a serious complication and major cause of mortality in hereditary haemochromatosis and beta thalassemia. Excessive iron in cardiomyocytes leads to oxidative damage of the myocardium. The mechanisms that accelerate the iron overloading in cardiomyocytes should be fully understood to adopt the modes of diagnosis and treatment in order to improve the quality of life in patients with hereditary haemochromatosis and  $\beta$ -thalassemia. This article gives a review of the possible mechanisms that increase the iron overload in cardiac cells, the different methods of diagnosis and the available treatments.

**Key Words:** Iron overload cardiomyopathy, hereditary haemochromatosis,  $\beta$ -thalassemia.**Corresponding Author:** Sairah Hafeez Kamran, University College of Pharmacy, University of the Punjab, Lahore, Pakistan. E-mail: [sairah.shahid@gmail.com](mailto:sairah.shahid@gmail.com)

Iron overloading can contribute to the development of disease in different ways because humans have no physiological pathway for the excretion of iron. 4g of iron is found in an average male adult. Each ml of packed erythrocytes contains about 1 mg i.e about more than 2g of iron is in the form of haemoglobin, 1g of iron is found in different body tissues predominantly liver and the rest of is present in iron containing proteins and myoglobin. The primary mechanism of regulation of concentration of iron in body depends on the absorption of iron. The pathological conditions due to iron overload in different vital organs such as heart arise due to increased iron absorption from the intestine which is the primary site for the regulation of iron haemostasis. The hormone that regulates the level of iron in body is a 25 amino acid peptide, hepcidin. Hepcidin inhibits the absorption of dietary iron and also regulates the release of iron from storage in hepatocytes. Hepcidin deficiency in body plays an important role in iron overload <sup>[1]</sup>. Iron overload mostly occurs due to genetic abnormalities like hereditary haemochromatosis. Excessive iron in the body can be result of blood transfusions especially transfusions in thalassemia <sup>[2]</sup>.

### **Iron overload Cardiomyopathy- General considerations**

One of the most critical complications associated with excessive iron in human body is cardiomyopathy. Cardiomyopathy weakens the force of cardiac muscle contractions thereby reducing the efficiency of blood circulation. Iron overload cardiomyopathy is a restrictive cardiomyopathy associated with systolic

and diastolic dysfunction and is considered secondary to the deposition of iron in cardiac muscles<sup>[3]</sup>. Excessive iron in tissues interferes with the ability of transferrin and ferritin protein to prevent the accumulation of free iron. In heart, iron overloading causes production of free radicals, superoxide and hydrogen peroxide, largely obtained from abnormal mitochondrial DNA<sup>[4]</sup>. The myocardial damage is largely associated with the production of reactive oxygen species, initiated by the large amounts of iron<sup>[5]</sup>. Excessive iron causes oxidative damage to the myocardial cells, membranes and mitochondrial respiratory chain enzyme<sup>[6]</sup>. Large amounts of iron bound to lipofuscin increase the sensitivity of lipofuscin loaded lysosomes to oxidative stress. Excessive accumulation of lipofuscin in myocardial cells causes defective lysosomal function. These defective lysosomes interferes with the normal autophagy and results in intracellular accumulation of aged and malfunctioning mitochondria, defective proteins and other waste products. These waste products have destructive effect on the performance and survival of cardiomyocytes. Lysosomal destabilization causes aging of myocardium and variety of cardiac pathologies<sup>[7]</sup>. Iron overload enhances the release of arachidonic acid and incorporation of arachidonic acid into phosphatidylcholine, as well as cyclooxygenase-2 induction and eicosanoid production, in neonatal rat ventricular myocytes. The effects of arachidonic acid and metabolites on cardiomyocyte rhythmicity suggest a connection between these signals and electromechanical alterations in iron-overload– induced cardiomyopathy. Increased release of arachidonic acid may contribute to progression of hypertrophy to heart failure in iron overload– induced cardiomyopathy<sup>[8]</sup>.

### **Clinical Presentation**

The patients with iron overload are usually asymptomatic in the early stages of disease. Because of the varied etiologies, symptoms may vary in different patients. Excessive iron overload in heart can result into irreversible heart failure if not detected earlier, so early identification of disease is very important. Initially a patient may experience shortness of breath because of exertion. This is usually due to left ventricular dysfunction resulting from restrictive pathophysiology. In cases of cardiac iron overload, iron first gets deposited in the epicardium and then progresses to endocardium and finally myocardium of the ventricle. The systolic function of the heart remains well preserved in the initial stages of the disease. In the later stages when iron deposition occurs in atrial tissue, AV blocks and spraventricular arrhythmias may occur. Deposition of iron in the conduction system gives arise to nodal disease causing bradyarrhythmias, therefore increasing the requirement for pacemaker placement. Left ventricular dilation and atrial fibrillation leads to the myocardial damage and this increases the risk of sudden cardiac death in iron overloaded patients<sup>[9]</sup>.

## Diagnosis

Over the last decade, cardiac magnetic resonance imaging has evolved as the most validated technique for the assessment of myocardial iron load and risk of cardiomyopathy in iron overload. In pathological states where excessive iron is present in the myocardial tissue, acts as a paramagnetic agent, producing changes in magnetic resonance signal intensity and susceptibility. Particularly it reduces magnetic resonance imaging relaxation times and leads to a reduction in the apparent myocardial T2\* value<sup>[10]</sup>.

The clinical grading for patients at risk of iron overload cardiomyopathy is based on cardiac T2\* values. These patients are divided into three categories.

Green zone= T2* >20 ms	These patients are at low risk for the imminent development of congestive heart failure.
Yellow zone= T2* between 10 and 20 ms	In these patients cardiac deposition has probably occurred are at intermediate risk of cardiac decompensation.
Red zone= T2* <10 ms	These patients are at high-risk of cardiac decompensation and need immediate attention and chelation therapy

[11]

## Treatments

The epidemiological and experimental evidences support the hypothesis that lowering iron levels in the body can decrease the risk of cardiovascular abnormalities. In order to remove excess iron from the body, iron chelation therapy must be given in order to reduce the chances of mortality induced by iron overload cardiomyopathy. The most commonly and widely used drug is deferoxamine<sup>[12]</sup>. In a randomized controlled trial it was observed that deferiprone improved right ventricular volume in a much better way when used as monotherapy as compared to deferoxamine<sup>[13]</sup>. Deferitron, hydroxybenzylethylenediamine-diacetic acid, desferrithiocin, pyridoxal isonicotinoyl hydrazone, 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone, and LINA-II (a deferiprone derivative) are among the newer chelating agents that are under active investigation. A drug with satisfactory oral bioavailability and good long-term efficacy and safety would be extremely useful, and a quest for such an agent is ongoing<sup>[14]</sup>. L type voltage gated calcium channel blockers (i.e amlodipine and verapamil) at therapeutic levels attenuated oxidative stress and myocardial iron accumulation. These drugs also prevent hypotension and also preserve heart structure and function. L type voltage gated calcium channel blockers provide protective function to the heart and reduce the iron overload in cardiomyopathy<sup>[15]</sup>. The diet plays an important role in decreasing the coronary events. It was observed that when milk whey protein was given to experimental murine model with iron supplementation, the mice receiving milk whey protein with iron had higher cardiac levels of glutathione peroxidase and glutathion than the mice treated with iron only and reduced levels of cytotoxic aldehydes<sup>[16]</sup>. Heart transplantation can be done in order to increase the survival rate and improve the quality of life. It can be done in combination with aggressive other suitable therapies to reduce the iron

overload. Caines et al. published a review in 2005 of 16 severe iron overload cardiomyopathic patients who received heart transplant from 1967-2003 with age ranging from 14-63 years. Three patients died in the first year due to development of infectious complications. Within thirty days mortality rate was 12%. 10 year survival rate was 41% with Kaplan-Meier analysis, with 1, 3 and 5 year survival rates of 81% [17].

The conditions largely associated with iron overload cardiomyopathy are genetic in nature. These include hereditary haemochromatosis and beta thalassaemia major.

### **Hereditary Haemochromatosis and Cardiomyopathy**

Hereditary haemochromatosis is an autosomal recessive disorder related to excessive iron in organs and results into organ damage. This disease was first described in 18<sup>th</sup> century because of the three major clinical indications of this disease i.e. cirrhosis, hyperpigmentation of the skin and diabetes, however only less than 15% patients present with this clinical triad. This disease is most exclusively found in populations of Northern Europe [18]. All these symptoms are related to increased and inappropriate absorption of iron. Excessive iron causes organ damage that leads to diabetes, cirrhosis and cardiomyopathies, hypogonadism and arthritis. Hereditary haemochromatosis is characterized by excessive absorption of iron from the diet taken and an increased recycling of iron by macrophages despite adequate available iron stores [19]. In 19<sup>th</sup> century it became clear that the disease is hereditary in nature and occurs due to a defect in gene residing on short arm of chromosome 6. The HFE gene was finally identified in 1996 [20]. Four types of inherited iron overload have been recognized:

**Type 1** is the most common form of hereditary haemochromatosis and is associated with mutations in the HFE gene on chromosome 6. The patients with type 1 usually have an autosomal recessive inheritance.

**Type 2** (juvenile hemochromatosis) is an autosomal recessive disorder. Mutations in this type are identified in the HJV gene (subtype A) on chromosome 1 and the HAMP gene (subtype B) on chromosome 19.

**Type 3** patients also have an autosomal recessive inheritance with mutations on chromosome 3 in the TfR2 gene.

**Type 4** is an autosomal dominant condition with heterozygous mutations on chromosome 2 in the ferroportin 1 gene [21].

In most cases of hereditary haemochromatosis it was seen that gene responsible for the expression of hepcidin becomes defective and results into the production of defective hepcidin. This defective hepcidin is unable to regulate the absorption and release of iron [18]. Pereira A.C., et al. in 2001 studied the distribution of haemochromatosis related mutations in 319 patients with heart failure due to cardiomyopathy of different etiologies. It was observed that patients with

ischemic cardiomyopathy were heterozygotes for the C282Y mutations. The D63 mutation was not found to be related to the ischemic cardiomyopathy [22]. Mitochondrial DNA damage is catalyzed by chronic iron overload and is associated with decreased expression of mitochondrial DNA that encodes mRNA and proteins in patients with haemochromatosis. This causes loss of mitochondrial respiratory capacity and leads directly to cardiac dysfunction. Mitochondrial DNA is more sensitive to oxidative damage as compared to the nuclear DNA. In haemochromatotic patients sudden heart failure and death occurs due to rapid deterioration of systolic function and one possibility of this could be mitochondrial failure arising from damage to mitochondrial DNA [4]. Oxidative damage is mediated by iron overload in the heart. The removal of superoxide radicals produced during oxidative damage is catalysed by manganese mitochondrial superoxide dismutase (MnSOD) in hereditary haemochromatosis patient. Valenti L. in 2004 observed that mice in which MnSOD gene has been knocked out develop fatal cardiomyopathy and this might be due to critical high mitochondrial concentration and oxygen tension in heart. In patients with hereditary haemochromatosis the presence of 16Val allele was associated with increased risk of cardiomyopathy. Patients with Val allele had higher dominance of cardiomyopathy than diabetes, cirrhosis or hypogonadism, independent of age, sex and alcohol misuse. The A16V mitochondrial superoxide dismutase polymorphism affects the risk of cardiomyopathy in hereditary haemochromatosis and is found to be associated with hereditary haemochromatosis [23]. In 2003 it was observed that if HFE gene is knocked out in mice, their hearts were found more susceptible to ischemia-reperfusion injury. This occurs due to increased myocardial infarct size, increased post ischemic ventricular dysfunction and cardiomyocyte apoptosis when compared with wild-type control hearts. The degree of injury increased in the hearts of the mice fed high-iron diet. The hearts of the HFE knockout mice showed increased content of reactive oxygen species and increased iron deposition in cardiomyocytes. Increased formation of malondialdehyde also increased the formation of reactive oxygen species and reduced antioxidant enzymes including glutathione peroxidase, catalase and superoxide dismutase [24].

### Clinical presentation

Intense transepithelial uptake of iron in patients with hereditary haemochromatosis leads to iron accumulation in body that results in pancreatitis, hepatocellular carcinoma, cirrhosis and cardiomyopathy. Two types of mutations are considered to be modulators of cardiovascular disease,

1. The first type of mutation causes substitution of tyrosine for cysteine at 282 amino acid position of protein product (cys282tyr C282Y).
2. The second type of mutation involves the substitution of aspartic acid for histidine at position 63 (his63asp, H63D).

Hereditary hemochromatosis genotypes C282Y/C282Y, C282Y/H63D, or C282Y/wild-type are risk factors for ischemic heart disease and myocardial infarction. These types of mutations are common in white population of North Europe, in whom 12% are heterozygous for C282Y and 24% are heterozygous for H63D [25-26]. Allen K.J, et al. assessed HFE mutations in 31,192 persons in Northern Europe between the ages of 40 and 69. The proportion of C282Y

homozygotes who had documented iron overload related disease were 28.4% men and 1.2% women. So iron overload disease in C282Y homozygotes is more prevalent in men than women [27].

### Diagnosis

The first step in the diagnosis of hereditary haemochromatosis is screening the transferrin saturation. If the fasting transferrin saturation in a patient is  $\geq 45\%$ , then serum ferritin levels should be tested. If the ferritin level is  $\geq 200 \mu\text{g/L}$  in premenopausal women or  $\geq 300 \mu\text{g/L}$  in postmenopausal women or in men then the possibility of hereditary haemochromatosis must strongly be considered. The next step in evaluation is genotyping. If a patient has elevated transferrin saturation and ferritin levels then genotyping must be recommended. If the patient is found to be homozygous for the C282Y mutation, presence of hereditary haemochromatosis is definite, and therapy should be initiated. In patients with hereditary haemochromatosis the life expectancy is reduced due to myocardial damage leading to cardiac complications and sudden cardiac death, but a case of sudden cardiac death in a young man was reported and it was seen that none of the known hereditary haemochromatosis mutations were present. This case suggests that genetic screening alone is not sufficient to identify the persons at risk of developing hereditary haemochromatosis [28]. This is because the genotypes (i.e., C282Y/H63D, C282Y/wild, or H63D/H63D) which are less clearly associated with clinical hereditary haemochromatosis and excessive iron indices diagnosis is more difficult to establish. In these types of patients further evaluation with liver biopsy might be necessary [29]. If diagnosis after liver biopsy remains in doubt, a trial of phlebotomy could be considered. It is generally clinically agreed that iron overload is present if 16-500 ml (equivalent to 4 g of iron) phlebotomies can be done without inducing iron deficiency [25].

### Treatment

The treatment for hereditary haemochromatosis from medieval times is phlebotomy i.e performing of periodic bleeding. Initially about 500-1000 ml of blood containing about 400-500 mg of iron are removed weekly until serum ferritin levels are reduced below 50 ng/ml and transferrin concentration is reduced to a value below 30% (requiring 2 to 3 years). Maintenance therapy during life with less aggressive bleeding is necessary to keep the transferrin saturation value below 50% and the serum ferritin levels less than 100 ng/ml. The quality of patients' lives is greatly affected because the rate of iron reloading is highly variable and the transferrin saturation remains elevated in many treated patients and does not normalize unless the patients become iron deficient. Gene therapy could alter specific targets to greatly reduce the accumulation of iron in haemochromatosis patients. The targets that have been studied so far include

1. reduction of the basolateral ferroportin transporter levels in enterocytes
2. reduction of the apical DMT-1 transporter levels in enterocytes
3. overexpression of the wild type HFE protein in enterocytes

4. overexpression of the iron uptake inhibitory protein hepcidin generated by hepatocytes.<sup>[30]</sup>

### **β-Thalassemia Cardiomyopathy**

The word “thalassemia” comes from a Greek word “thalassa” meaning sea. The disease is highly prevalent in the areas bordering the sea like Middle East, North India, Southeast Asia and Mediterranean Basin. Thalassemia is the most common genetic disorder that causes decreased globin, protein composition of haemoglobin. Two clinical forms of thalassemia have been identified, β-thalassemia major and thalassemia intermedia. Beta thalassemia major (TM) is the severe form of genetic disorder arising either from homozygous or compound heterozygous defects. TM requires repetitive blood transfusions because of severe anemia that arises from the defective erythropoiesis. Repetitive transfusions increase iron content in body<sup>[31]</sup>.

### **Clinical presentation**

Two thirds of deaths in β-thalassemic patients occur due to heart failure. The pathogenesis of cardiac failure in β-thalassemia is complex and is thought to be linked to the viral infections and immunogenetic factors<sup>[32]</sup>. Iron overload cardiomyopathy however doesn't occur in the initial stages of disease and the patient is usually asymptomatic, but is considered as the most serious complication. Iron overload cardiomyopathy usually begins when 20g or more iron gets accumulated and no iron chelation therapy is given. Restrictive cardiomyopathy usually occurs first, followed by dilated cardiomyopathy<sup>[33]</sup>. Diastolic dysfunction occurs prior to the systolic dysfunction and heart failure. Left side heart failure is more common than right side failure. In a group of 52 patients with thalassemia major, the mean age of the patients diagnosed with heart failure was 25±5 years. These 52 patients were given repetitive blood transfusions, regular iron chelation therapy and conventional heart failure therapy and it was seen that 5 year survival rate was 48%<sup>[34]</sup>. In a cohort study of 202 well treated patients with thalassemia major the prevalence of cardiac failure was 2.5% with a mean age of 27±6 years. Of these 2.5%, 5% had a history of acute pericarditis<sup>[35]</sup>. In patients with thalassemia major without heart failure the HLA-DRB1\*1401 allele was found more frequently, whereas the HLA-DQA1\*0501 allele was found more frequent in patients with heart failure. This suggests that the HLA-DQA1\*0501 allele might be related to an increased risk for heart failure, whereas the HLA-DRB1\*1401 allele might be protective against heart failure<sup>[31]</sup>.

### **Diagnosis**

The diagnosis of iron overload in thalassemic patients has until recently been carried out by using ferritin levels and liver iron concentrations as surrogate markers and echocardiography. Generally it was observed that the patients have developed cardiomyopathy by the time changes are seen in echocardiography. However determining certain parameters in echocardiography such as Total diameter index can predict cardiac iron overload. This method is highly specific for determining cardiac iron overload but has low sensitivity. Most recent study reveals the significance of cardiac magnetic resonance imaging technique in assessing the cardiac iron overload and identifying thalassemia patients at the risk of developing cardiac disease<sup>[36]</sup>.

Cardiac magnetic resonance imaging also helps in tailoring the therapies for removal excess iron. The iron uptake mechanisms were studied in cultured thalassemic cardiomyocytes model. T type calcium channels pathway was found responsible for the uptake of iron in cultured thalassemic cardiomyocytes so T type calcium channel blockers could also prevent iron uptake in cultured thalassemic cardiomyocytes. L-type calcium channel blockers however could not prevent the uptake of iron in  $\beta$ -thalassemia patient<sup>[37]</sup>.

### Treatment

In beta thalassemic patients iron chelation therapy is very important in saving lives because prior to the introduction of chelating therapy most patients did not reach to the second decade of life, mainly owing to heart diseases. Desferrioxamine, deferiprone and deferasirox are iron chelators currently available. Desferrioxamine is now not very much preferred due to poor compliance. Deferasirox is a new iron chelator orally bioavailable. Deferasirox protects the cells from non transferrin bound serum iron and plasma iron. Plasma iron is mainly responsible for generating reactive oxygen species which is responsible for damaging cells. The oral iron chelators have better compliance because of oral use. These three chelators when used properly will improve the prevention and treatment of iron overload and improve the quality of life of patients receiving transfusions<sup>[38]</sup>. Deferiprone (>80 mg/kg/day) is found to be effective in the removal of cardiac iron, in the reversal of iron overload related cardiomyopathy, in the maintenance of normal iron stores and the overall long-term survival of thalassemia patients<sup>[39]</sup>. A 15-year-old male with beta-thalassemia major developed dilated cardiomyopathy secondary to iron overload. This patient was treated with deferoxamine but the therapy was unsuccessful, presumably due to poor compliance. Deferasirox was then given for 15 months and it was observed that left ventricular end-diastolic dimension normalized, and ejection fraction improved to 58%. Treatment with deferasirox resulted in a reversal of iron-induced cardiomyopathy in thalassemic patients<sup>[40]</sup>. In 2006, first human gene therapy trial was done in France for sickle cell disease and thalassemia. The first patient treated failed for technical reasons because he required back up of his own thalassemic bone marrow. In 2007, an 18 year old patient was transplanted and has been recently reported to be free of transfusions after 40 months of post-transplant follow up<sup>[41]</sup>. It is hoped that introduction of healthy hematopoietic stem cells in severe congenital anemia lacking appropriate  $\beta$ -hemoglobin production, such as in  $\beta$ -thalassemia, may reverse the primary pathophysiology in the disease and reduce the need for transfusions. Stem cell transplantation requires chemotherapy and radiation techniques to make it successful if perfectly matched donors are not available. Presently, clinical trials are underway to test low-intensity radiation along with immunosuppressant drugs without chemotherapy to accomplish successful stem cell transplantation with a half-matched donor in order to broaden its use<sup>[42]</sup>.

### CONCLUSION

Iron overload is a serious complication of hereditary haemochromatosis and  $\beta$ -thalassemia. Early diagnosis can make this complication less serious and easily treatable and this can be accomplished with cardiac imaging of iron quantity in cardiomyocytes. The mechanisms of iron homeostasis and the uptake of iron by cardiomyocytes are emerging and elucidated to some extent to understand the underlying possibilities in order to adopt the best possible treatment.



Newer chelating agents are being introduced to increase the compliance of patients. Heart transplant, gene therapy and stem cells techniques must further be investigated to increase the survival rate and more sophisticated methods be developed to improve the quality of life of patients.

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